Volume 10, [illegible] October, 1937

The Yale Journal of Biology and Medicine

141 [illegible]

[illegible] Robert E. Handschu

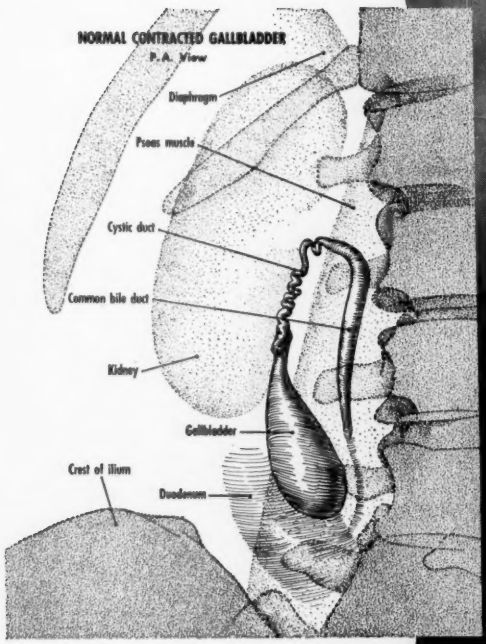
[illegible]

[illegible] Josef E. [illegible]

[illegible]

[illegible]

[illegible]



"There are three main advantages in the use of

Telepaque®

SUPERIOR ORAL CHOLECYSTOGRAPHY AND CHOLANGIOGRAPHY

1. **Excellent cholecystograms are readily obtainable.**
2. **The side reactions are usually minimal, only rarely very disturbing, and often completely absent.**
3. **In a fairly large percentage of cases, the cystic and the common ducts are quite definitely outlined, and occasionally even the hepatic duct."**

Buckstein, Jacob: The Digestive Tract in Roentgenology. Philadelphia, J. B. Lippincott Co., 2nd ed., 1953, vol. 2, p. 1003.

DOSAGE:

The average adult dose of Telepaque is 3 Gm. (6 tablets). In persons of thin or medium build, weighing less than 150 lb., 2 Gm. (4 tablets) may be sufficient.

SUPPLIED:

Tablets of 0.5 Gm. in envelopes of 6 tablets, boxes of 5 and 25 envelopes, and bottles of 500.

Winthrop LABORATORIES

NEW YORK 18, N.Y. • WINDSOR, ONT.

Telepaque (brand of iopanoic acid), trademark reg. U. S. Pat. Off.

CONTENTS

161 **HAROLD SAXTON BURR**

163 **BIBLIOGRAPHY OF HAROLD SAXTON BURR**

168 **STUDIES ON THE CARCINOSTATIC ACTIVITY IN MICE OF 6-AZAURACIL RIBOSIDE (AZAURIDINE), IN COMPARISON WITH THAT OF 6-AZAURACIL** *Julian J. Jaffe, Robert E. Handschumacher, and Arnold D. Welch*

176 **VISCERAL MANIFESTATIONS OF EPILEPSY** *Gilbert H. Glaser*

187 **HISTORY OF PULMONARY RESECTION** *Gustaf E. Lindskog*

201 **SOME UNEXPLAINED METABOLIC ACTIONS OF PITUITARY HORMONES WITH A UNIFYING HYPOTHESIS CONCERNING THEIR SIGNIFICANCE** *Frank L. Engel*

224 **KINETICS OF MUSCULAR CONTRACTION—I** *M. C. Goodall*

248 **BOOK REVIEWS**

244 **EVENTS**

255 **ADDITIONAL BOOKS RECEIVED**

Telepaque (brand of iopanoic acid), trademark reg. U. S. Pat. Off.

THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

Founded in 1928.

George H. Smith: *Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-52.*

BOARD OF EDITORS

Faculty

Paul B. Beeson; Frederick G. Kilgour, *Managing Editor;* James W. Lewis, *Advertising Manager;* Averill A. Liebow; Vernon W. Lippard, *Chairman;* C. N. H. Long; Roy Schafer; Max Taffel; Elizabeth H. Thomson, *Assistant Editor;* Talbot H. Waterman

Students

Roger P. Atwood; Neil R. Cooper; Malin R. Dollinger; Stanley I. Harris; William S. Kaden; Myron M. Lotz; N. Ronald Morris; Robert S. Neuwirth; James A. O'Neill, Jr.; David W. E. Smith; Lisa A. Steiner; George E. Watson, 3d

---

Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

Kodak reports on:

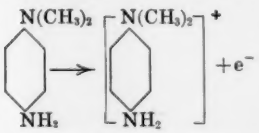
oxidation and the ills of the spirit . . . a high level of aberration correction at popular prices

Crazy!

During the past spring we noted a sharp upturn in the volume of our correspondence with psychiatrists. They all wanted to know whether we could supply the compound N,N-dimethyl-p-phenylenediamine dihydrochloride. In accordance with practice in the retailing game when you don't have exactly what the customer asks for, we replied to each that we would be happy to supply the monohydrochloride at $2.20 for 25 grams. This rejoinder got us nowhere.

Thus rebuffed (and deservedly so), we dipped a little into the fast-breaking literature of the borderland between chemistry and the ills of the spirit.

We learned about ceruloplasmin. It's an oxidation-promoting enzyme, a protein that accounts for most of the copper in the body. In *Science* for January 18, 1957 (*125*,117) appeared evidence that ceruloplasmin runs high in serum of schizophrenic patients, as adduced from an increased rate and depth of color development in the *in vitro* reaction



Here we found a region with landmarks familiar indeed to anyone who has thought deeply about photographic developers. p-Phenylenediamine is parent to a numerous family of aromatic amines that assume color when oxidized. Stability is enhanced by various substitutions and in the dry crystalline state by conversion to some salt or other.

If, in this case, the author chose to have two methyl groups on one of the amine nitrogens and, instead of one, two molecules of HCl to preserve his reagent on the shelf until ready for use, that was his privilege. So also is it the inalienable right of any neurophysiologist who wishes to pursue this line of laboratory investigation to be able to buy this dihydrochloride ready made without bothering to add the appropriate quantity of HCl to a solution of the base or the monohydrochloride. (The right may be exercised by purchase from us of *N,N-Dimethyl-p-phenylenediamine Dihydrochloride* (Eastman 7423) at $3.05 for 25 grams.)

And now, alongside one group of wise men who explain the tension of the times in terms of socio-economic forces and another group of wise men who watch the war between the id, the ego, and the superego, there stands huddled in stimulating talk a new group of wise men who point out that the tranquillizing chlorpromazine and Rauwolfia alkaloids are potent oxidase inhibitors. But even they seem largely to agree that anybody who at this time would depend solely on these enzymatic measurements for a positive diagnosis of schizophrenia is crazy.

"Eastman Organic Chemicals, List No. 40" contains a long catalog of many substances other than ceruloplasmin for which we offer reagents and abstracts on their use. This alone is worth the space in your bookcase. For a copy of List No. 40 (which also catalogs some 3600 available compounds) write Distillation Products Industries, Eastman Organic Chemicals Department, Rochester 3, N. Y. (Division of Eastman Kodak Company).

The boon of lanthanum

Our former research vice president, Dr. C. E. Kenneth Mees, one of a few men who shortly before World War I conceived the novel idea that science had a place in industry, has suggested to us from his home in Honolulu that the public ought to be told more about rare element glass.

Very well. Along about 1934, as a result of some rather deep studies in glass chemistry, we found that by replacing certain traditional glass ingredients with such oddities as lanthanum oxide, one could make a glass of very high index but with a dispersion low enough for use in positive elements. The higher the index, the shallower the curves can be. Each surface in a lens system contributes aberrations, both plus and minus. The deeper the curve, the bigger they are. The art of lens design consists of playing them off against each other. The more surfaces, the better the attainable correction.

Before long, Kodak lenses demanding the best possible performance were being put out with elements of the new glass, regardless of the cost of lanthanum and of 10-pound batch production in pure platinum crucibles.

Now our philosophy goes like this: Shallow curves not only introduce less aberrations, but geometry permits more of them on a single block for grinding and polishing. This economy can pay for a pretty high glass cost. At the same time, the customer gets a level of aberration correction superior to what the same number of components could have bought him before lanthanum.

Doctor Mees and we hope that when next you shop for a personal camera, you give particular consideration to the Kodak Pony 135, *the* Kodak Pony IV, *the* Kodak Signet 30, 40, *and* 50 Cameras, *and the fixed f/1.9 lens of the* Cine-Kodak Medallion 8 Camera. *They've got it. Lanthanum, that is.*

Prices quoted are subject to change without notice.

This is another advertisement where Eastman Kodak Company probes at random for mutual interests and occasionally a little revenue from those whose work has something to do with science

Kodak
TRADEMARK

Courtesy of the Yale University Art Gallery

HAROLD SAXTON BURR

From a painting by Artzybasheff presented to the Yale University School of Medicine on December 6, 1957 by the Burr Portrait Committee on behalf of Dr. Burr's colleagues and former students.

HAROLD SAXTON BURR

On January 1, 1958, Harold Saxton Burr, E. K. Hunt Professor of Anatomy, retires after more than forty-three years as a member of the faculty of the Yale University School of Medicine. Anatomist, artist, and teacher, he has influenced many widely differing groups of people.

Born in Lowell, Massachusetts, on April 10, 1889, Dr. Burr is of old Connecticut stock. His father was Hanford M. Burr of Lyme, and his mother Clara H. Saxton of Westport. He attended the public schools and, in 1907, graduated from the Technical High School of Springfield, Massachusetts. The next year he attended Springfield College, where his father was Professor of Economics.

In the autumn of 1908 he attended the Sheffield Scientific School at Yale and received his Ph.B. degree in 1911. He immediately began graduate work under Professor Ross G. Harrison and was granted the Ph.D. degree in 1915.

In 1914 Burr became Instructor in Anatomy with Dr. Harry Burr Ferris in the School of Medicine, being promoted to an Assistant Professorship in 1919, to Associate Professor in 1926, and to Professor in 1929. On the retirement of Dr. Ferris in 1933, Dr. Burr became Acting Head of the Department, was appointed to the E. K. Hunt Professorship of Anatomy, and was placed in charge of the Section of Neuro-Anatomy. Following Dr. Edgar Allen's death in 1943, Dr. Burr was again, briefly, Acting Head of the Department.

Having entered Yale in 1908, Dr. Burr has a record of affiliation with the University of nearly fifty years. He is, however, not the only member of his family to establish a long connection with one institution. His grandfather, Enoch Fitch Burr, of the Yale Class of 1839, served as pastor of the First Congregational Church of Lyme from 1850 to 1907!

As an anatomist, Dr. Burr has published, either alone or with others, some 93 papers and has stimulated his associates in the Section of Neuro-Anatomy in the production of about a hundred more. His own earlier contributions dealt chiefly with the development of the central nervous system. Later, he studied various aspects of the development of peripheral nerves. These were the results of his experimental embryological investigations. In 1939 he became interested in electrodynamic processes. From that time to the present his studies have centered mainly about the question of bioelectric potentials in animals and plants. In the human organism his studies concerned the bioelectric phenomena of ovulation and menstruation. A number of his clinical colleagues collaborated in various of the papers resulting from this work, as they also did in his studies of cancerous tumors. In the broader philosophical aspects of an electrodynamic theory of life

phenomena, Professor F. S. C. Northrop was a collaborator. Woven through Burr's scientific and philosophical contributions are a number on subjects in the realm of the history of medicine.

It is interesting to visit Dr. Burr in his ancestral home, "Mansewood," in Lyme and to discover various trees hooked up to recording voltmeters. They tell the story of their electrical potential changes in fair weather and storm.

As an artist, Dr. Burr has brought much pleasure to others and to himself. While in Springfield he had studied painting with William Whitmore. Concentrating largely on landscapes, with the Lyme region of Connecticut as the source of much of his inspiration, Saxton Burr, as he signed many of his paintings, gives proof of his very real talent in interpreting the moods of the seasons in woods, meadow, and shore. Long a member of the Lyme Art Association, he has been an unfailing exhibitor at their exhibitions. Many of his paintings have been sold and the products of his brush adorn homes throughout the country.

As a teacher, Dr. Burr has made a decided impact on his students. Those more interested and gifted found his teaching inspiring and he has stimulated not a few to go into clinical neurology and neurosurgery, as well as into morphological neuroanatomy. Less interested students found his instruction difficult, for neuroanatomy is no sinecure and demands a certain flair. However, the secretary of the Burr Portrait Committee reports that the appreciation and affectionate regard expressed by many of his former students give enviable testimony to the success of their teacher, for such comments are granted to few.

Dr. Burr is, of course, a member, associate, or fellow of a number of scientific and medical societies, including the American Association of Anatomists, which he served as a member of its Executive Committee from 1929 to 1933, The Association for Research in Nervous and Mental Disease, The American Neurological Association, the Connecticut State Medical Society, and the American Association for the Advancement of Science. He is also a member of the Beaumont, the History, and the Nathan Smith Clubs, to each of which he has contributed historical papers, and of Sigma Xi. He has been a Fellow of Silliman College since its organization when the late Charles Hyde Warren was its Master. Dr. Burr likewise served for many years as a member of the Editorial Board of the Yale Journal.

Hal Burr has not foresworn his civic duty. He is a Deacon of the First Congregational Church of Lyme, the same church of which his grandfather

was so long the pastor. In politics, he is a Republican and has just been elected to the Lyme School Board.

On December 27, 1911, Harold Saxton Burr and Jean Forrest Chandler were married. In 1919, their son, Peter Saxton, was born. He and his attractive wife, daughter, and son now spend the summers with Grandma and Grandpa Burr in Lyme. The old homestead has been modernized within, except for the oldest part of the house, pre-dating the Revolution, which is now the Burr "Tap Room." Here their guests are royally entertained. The Burrs have lived in Lyme for the past few years and here they will continue to dwell in retirement, which will be that in name only, for neither Hal nor Jean will ever be inactive. May they live long and prosper!

D. H.

BIBLIOGRAPHY OF HAROLD SAXTON BURR

1916

1. The effects of the removal of the nasal pits in Amblystoma embryos. *J. exp. Zool.*, 1916, *20*, 2757.
2. Regeneration in the brain of Amblystoma. *J. comp. Neurol.*, 1916, *26*, 203-211.

1918

3. Breeding habits, maturation of eggs and ovulation of the albino rat. *Amer. J. Anat.*, 1918, *15*, 291-317.

1920

4. The transplantation of the cerebral hemispheres of Amblystoma. *J. exp. Zool.*, 1920, *30*, 159-169.

1922

5. The early development of the cerebral hemispheres in Amblystoma. *J. comp. Neurol.*, 1922, *34*, 277-301.

1924

6. An experimental study of the origin of the meninges. *Proc. Soc. exp. Biol. (N. Y.)*, 1924, *22*, 52-53 (with S. C. Harvey).
7. Some experiments on the transplantation of the olfactory placode in Amblystoma. 1. An experimentally produced aberrant cranial nerve. *J. comp. Neurol.*, 1924, *37*, 455-479.

1925

8. An anatomical study of the gasserian ganglion, with particular reference to the nature and extent of Meckel's cave. *Anat. Rec.*, 1925, *29*, 269-289 (with G. B. Robinson).

1926

9. An experimental study of the action of hyoscine hydrobromide on the nervous system of Amblystoma. *J. comp. Neurol.*, 1926, *41*, 401-421.
10. The development of the meninges. *Arch. Neurol. Psychiat.*, 1926, *15*, 545-565 (with S. C. Harvey).

1928

11. Certain factors determining the direction of growth of nerve fibers. *Science,* 1928, *74,* 604.
12. The central nervous system of Orthagoriscus mola. *J. comp. Neurol.,* 1928, *45,* 33-128.

1929

13. Jonathan Knight and the founding of the Yale Medical School. *Yale J. Biol. Med.,* 1929, *1,* 327-343.

1930

14. Hyperplasia in the brain of Amblystoma. *J. exp. Zool.,* 1930, *55,* 171-191.
15. Disciplines. *Yale J. Biol. Med.,* 1930, *3,* 151-157.

1931

16. The preclinical sciences and human biology. *Yale J. Biol. Med.,* 1931, *4,* 63-68.
17. Development of the meninges in the chick. *Proc. Soc. exp. Biol. (N. Y.),* 1931, *28,* 974-976 (with S. C. Harvey).

1932

18. Determinants of organization in the cerebral hemispheres. *Res. Publ. Ass. nerv. ment. Dis.,* 1932, *13,* 39-48.
19. An electro-dynamic theory of development suggested by studies of proliferation rates in the brain of Amblystoma. *J. comp. Neurol.,* 1932, *56,* 347-371.

1933

20. Development of the meninges. *Arch. Neurol. Psychiat.,* 1933, *29,* 683-690 (with S. C. Harvey).

1934

21. The founding of the Medical Institution of Yale College. *Yale J. Biol. Med.,* 1934, *6,* 333-340.
22. Some observations on the neural mechanisms of Opisthoproctus Soleatus Vaillant. *Psychiat. neurol. Bl.,* 1934, *38,* 407-416 (with G. M. Smith).
23. Sympathetic components of the genito-femoral and obturator nerves in the Rhesus monkey (Macaca mulatta). *Anat. Rec.,* 1934, *61,* 53-56 (with S. Zuckerman).

1935

24. A study of the effects of intermedin and injury of the hypophysis on traumatic corial melanophores in goldfishes. *Endocrinology,* 1935, *19,* 409-412 (with G. M. Smith and R. S. Ferguson).
25. The electro-dynamic theory of life. *Quart. Rev. Biol.,* 1935, *10,* 322-333 (with F. S. C. Northrop).
26. Electrical characteristics of living systems. *Yale J. Biol. Med.,* 1935, *8,* 31-35 (with C. T. Lane).
27. Detection of ovulation in the intact rabbit. *Proc. Soc. exp. Biol. (N. Y.),* 1935, *33,* 109-111 (with R. T. Hill and Edgar Allen).

1936

28. Electro-dynamic studies of mice with developing cancer of the mammary gland. *Anat. Rec.,* 1936, *64,* 7-8.
29. A vacuum tube microvoltmeter for the measurement of bio-electric phenomena. *Yale J. Biol. Med.,* 1936, *9,* 65-76 (with C. T. Lane and L. F. Nims).
30. Bio-electric phenomena associated with menstruation. *Yale J. Biol. Med.,* 1936, *9,* 155-158 (with L. K. Musselman).

1937

31. Experimental findings concerning the electrodynamic theory of life and an analysis of their physical meaning. *Growth*, 1937, *1*, 78-88 (with F. S. C. Northrop).
32. Bio-electric potential gradients in the chick. *Yale J. Biol. Med.*, 1937, *9*, 247-258 (with C. I. Hovland).
33. Bio-electric correlates of development in Amblystoma. *Yale J. Biol. Med.*, 1937, *9*, 541-549 (with C. I. Hovland).
34. Medical fees in the colonial period. *Yale J. Biol. Med.*, 1937, *9*, 359-364.
35. A bio-electric record of human ovulation. *Science*, 1937, *86*, 312 (with L. K. Musselman, D. Barton, and N. Kelley).
36. Bio-electric correlates of human ovulation. *Yale J. Biol. Med.*, 1937, *10*, 155-160 (with L. K. Musselman, D. Barton, and N. Kelley).

1938

37. Steady-state electrical properties of the human organism during sleep. *Yale J. Biol. Med.*, 1938, *10*, 271-274 (with D. S. Barton).
38. Bio-electric properties of cancer-resistant and cancer-susceptible mice. *Amer. J. Cancer*, 1938, *32*, 240-248 (with L. C. Strong and G. M. Smith).
39. Bio-electric correlates of the menstrual cycle in women. *Amer. J. Obstet. Gynec.*, 1938, *35*, 743-751 (with L. K. Musselman).
40. Bio-electric correlates of methylcolanthrene-induced tumors in mice. *Yale J. Biol. Med.*, 1938, *10*, 539-544 (with L. C. Strong and G. M. Smith).
41. The relationship between the bio-electric potential of rats and certain drugs. *Yale J. Biol. Med.*, 1938, *11*, 137-140 (with P. K. Smith).
42. The measurement of pH in circulating blood. *Science*, 1938, *87*, 197-198 (with L. F. Nims and Clyde Marshall).
43. Bio-electric correlates of wound healing. *Yale J. Biol. Med.*, 1938, *11*, 104-107 (with S. C. Harvey and Max Taffel).

1939

44. Evidence for the existence of an electro-dynamic field in living organisms. *Proc. nat. Acad. Sci.*, 1939, *25*, 284-288 (with F. S. C. Northrop).
45. A preliminary study of electrical correlates of growth in Obelia geniculata. *Growth*, 1939, *3*, 211-220 (with Fred S. Hammett).
46. Voltage gradients in the nervous system. *Trans. Amer. neurol. Ass.*, 1939, *65*, 11-14 (with P. J. Harman, Jr.).
47. Vocationalism in the university. *Yale J. Biol. Med.*, 1939, *12*, 199-204.
48. Animal electricity. *Yale scient. Mag.*, 1939, *13*, 5.

1940

49. Biologic organization and the cancer problem. *Yale J. Biol. Med.*, 1940, *12*, 277-282.
50. Vaginal electrical correlates of the estrous cycle of the rat. *Anat. Rec.*, 1954, *76*, 8 (with John Boling and D. Barton).
51. An electrometric study of the healing wound in man. *Yale J. Biol. Med.*, 1940, *12*, 483-485 (with Max Taffel and S. C. Harvey).
52. Harry Burr Ferris. *Science*, 1940, *92*, 499-500.
53. Electrometric studies of tumors induced in mice by the external application of benzpyrene. *Yale J. Biol. Med.*, 1940, *12*, 711-717 (with G. M. Smith and L. C. Strong).

1941

54. Factors associated with vaginal electrical correlates of the estrous cycle of the albino rat. *Anat. Rec.*, 1941, *79*, 9 (with John Boling).

55. Field properties of the developing frog's egg. *Proc. nat. Acad. Sci.*, 1941, *27*, 276-281.
56. Changes in the field properties of mice with transplanted tumors. *Yale J. Biol. Med.*, 1941, *13*, 783-788.
57. An electrometric study of uterine activity. *Amer. J. Obstet. Gynec.*, 1941, *42*, 59-67 (with L. Langman).
58. Steady state potential differences in the early development of Amblystoma. *Yale J. Biol. Med.*, 1941, *14*, 51-57 (with T. H. Bullock).

1942

59. An electrical study of the human cervix uteri. *Anat. Rec.*, 1942, *82*, 35-36 (with L. Langman).
60. Electrical correlates of growth in corn roots. *Yale J. Biol. Med.*, 1942, *14*, 581-588.
61. Electrometric timing of human ovulation. *Amer. J. Obstet. Gynec.*, 1942, *44*, 223-230 (with L. Langman).

1943

62. Neuroid transmission in mimosa. *Anat. Rec.*, 1943, *85*, 12.
63. Electrical correlates of pure and hybrid strains of corn. *Proc. nat. Acad. Sci.*, 1943, *29*, 163-166.
64. Electrical polarization of pacemaker neurons. *J. Neurophysiol.*, 1943, *6*, 85-97 (with T. H. Bullock).
65. An electrometric study of mimosa. *Yale J. Biol. Med.*, 1943, *15*, 823-829.

1944

66. Moon-madness. *Yale J. Biol. Med.*, 1944, *16*, 249-256.
67. A biologist considers social security. *Conn. St. med. J.*, 1944, *8*, 165.
68. Potential gradients in living systems and their measurements. Pp. 1117-1121 in *Medical Physics*. Chicago, The Year Book Publishers, Inc. [c1944].
69. The meaning of bio-electric potentials. *Yale J. Biol. Med.*, 1944, *16*, 353-360.
70. Electric correlates of form in Cucurbit fruits. *Amer. J. Botany*, 1944, *31*, 249-253 (with E. W. Sinnott).
71. Electricity and life: phases of moon correlated with life cycle. *Yale scient. Mag.*, 1944, *18*, 5-6.

1945

72. Variables in DC measurement. *Yale J. Biol. Med.*, 1945, *17*, 465-478.
73. Diurnal potentials in the maple tree. *Yale J. Biol. Med.*, 1945, *17*, 727-734.

1946

74. Electrical correlates of peripheral nerve injury. A preliminary note. *Science*, 1946, *103*, 48-49 (with R. G. Grenell).
75. Growth correlates of electromotive forces in maize seeds. *Proc. nat. Acad. Sci.*, 1946, *32*, 73-84 (with Oliver Nelson, Jr.).
76. Surface potentials and peripheral nerve injury: A clinical test. *Yale J. Biol. Med.*, 1946, *18*, 517-525 (with R. G. Grenell).

1947

77. Tree potentials. *Yale J. Biol. Med.*, 1947, *19*, 311-318.
78. Electromagnetic studies in women with malignancy of cervix uteri. *Science*, 1947, *105*, 209-210 (with L. Langman).
79. Field theory in biology. *Sci. Monthly*, 1947, *64*, 217-225.
80. Surface potentials and peripheral nerve regeneration. *Fed. Proc.*, 1947, *6*, 117 (with R. G. Grenell).

1948

81. A commentary on full-time teaching. *Conn. St. med. J.*, 1948, *12*, 937-940.

1949

82. Millivoltmeters. *Yale J. Biol. Med.*, 1949, *21*, 249-253 (with A. Mauro).
83. A technique to aid in the detection of malignancy of the female genital tract. *Amer. J. Obstet. Gynec.*, 1949, *57*, 274-281.
84. Electrostatic fields of the sciatic nerve in the frog. *Yale J. Biol. Med.*, 1949, *21*, 455-462 (with A. Mauro).
85. The Connecticut State Medical Society. *Conn. St. med. J.*, 1949, *13*, 950-955.

1950

86. Electrometric study of cotton seeds. *J. exp. Zool.*, 1950, *113*, 201-210.
87. Bioelectricity: potential gradients. Pp. 90-94 in *Medical Physics*. Chicago, The Year Book Publishers, Inc., 1950.

1952

88. Electrometrics of atypical growth. *Yale J. Biol. Med.*, 1952, *25*, 67-75.

1953

89. Electrical correlates of ovulation in the rhesus monkey. *Yale J. Biol. Med.*, 1953, *25*, 408-417 (with V. L. Gott).

1954

90. Confusion or configuration? *The Astronomical League Bulletin*, 1954, *4*, No. 4.

1955

91. Certain electrical properties of the slime mold. *J. exp. Zool.*, 1955, *129*, 327-342.
92. Response of the slime mold to electric stimulus. *Science*, 1955, *122*, 1020-1021 (with William Seifriz).

1956

93. Effect of a severe storm on electric properties of a tree and the earth. *Science*, 1956, *124*, 1204-1205.

JULIAN J. JAFFE*
ROBERT E. HANDSCHUMACHER**
ARNOLD D. WELCH†

Department of Pharmacology, Yale University School of Medicine

STUDIES ON THE CARCINOSTATIC ACTIVITY IN MICE OF 6-AZAURACIL RIBOSIDE (AZAURIDINE), IN COMPARISON WITH THAT OF 6-AZAURACIL‡

INTRODUCTION

In the animal body the formation of new cells requires the synthesis of nucleic acids and for this purpose certain derivatives of pyrimidine bases (e.g., uracil, cytosine, and thymine) must be synthesized by, or supplied to, the parent cells. Accordingly, it would be reasonable to assume that significant interference with the formation or utilization of these pyrimidine-containing compounds could profoundly affect the rate at which new cells are formed in various tissues. It is often assumed that a potent inhibitor of cellular reproduction should affect all tissues in a comparable manner; however, many factors influence to a variable degree the capacity of a compound to disturb the reproduction of various types of cells in a variety of tissues. Indeed, the moderate degree of selectivity already observed with certain well-recognized carcinostatic agents is indicative of significant variability, in different loci, in such factors as transport into cells, affinity for those enzymes which normally react with physiological intermediates, and the degree of metabolic alteration of such agents.

The results of earlier studies from these laboratories of the biological effects of analogs of the naturally occurring pyrimidines, orotic acid,[9, 12, 13] thymine, and its deoxyriboside,[16, 17, 18] encouraged us to attempt to obtain a compound capable of interfering specifically with the metabolism of uracil-containing substances. Such a compound (or appropriate derivatives of it) could interfere not only with the synthesis of ribonucleic acids, but also with that of deoxyribonucleic acids, and with a sizable group of coenzymes containing either uracil or its derivative, cytosine.

---

* Instructor in Pharmacology.

** Assistant Professor of Pharmacology and Scholar in Cancer Research of the American Cancer Society.

† Eugene Higgins Professor of Pharmacology.

‡ This investigation was supported by a grant (C-2817) from the National Cancer Institute of the U. S. Public Health Service.

Received for publication November 26, 1957.

For this purpose a most promising possibility, as suggested by earlier studies of 6-azathymine (6-methyl-*as*-triazine-3,5-dione) and its deoxyriboside,[16, 17, 18] was a comparable analog of uracil, namely, 6-azauracil (*as*-triazine-3,5-dione), a substance with chemical structure and stability which made pertinent its biological investigation. Although prepared in 1947 for use in a study of chemical reaction mechanisms, the method of synthesis was not described in detail,[20] and, therefore, a new procedure was evolved.[3, 4]

Studies by Handschumacher and Welch[11] of the effect of 6-azauracil (AzU) on a variety of microorganisms indicated that this analog is an effective inhibitor of cellular reproduction and that its action under certain conditions can be nullified by the natural metabolites: uracil and uridine (uracil riboside). Of especial significance to further applications of the analog was the finding that the utilization not only of exogenous, but also of endogenously formed derivatives of uracil was blocked by the antagonist. It has been shown that with *Streptococcus faecalis* 8043, an organism highly susceptible to inhibition by AzU, drug-resistant mutants are selected by AzU.[11] The mechanism of this resistance is attributed[5] to the fact that among a significant population of *S. faecalis* cells there are individuals which possess no capacity to utilize free uracil and thus offer no pathway for the assimilation of AzU. Since such AzU-resistant cells can utilize uridine, it was reasoned that biochemical circumvention of this type of drug-resistance should be possible through the use of AzU-riboside (AzUR). This compound, azauridine, was prepared both biologically and chemically[9] and shown to be highly inhibitory to AzU-resistant bacteria. It should be mentioned, however, that resistance to AzUR also can be encountered in bacterial populations. Evidence that AzU enters the same pathways of metabolism as does uracil or uridine has been shown by the isolation from the acid-soluble fraction of microorganisms exposed to AzU-C^{14} of radioactive azauridine and its mono-, di-, and tri-phosphates; in addition, other derivatives of AzU are formed in small amounts.[9, 10]

Tests of AzU in mice bearing various transplantable neoplasms, carried out collaboratively at Yale and the National Cancer Institute (Law), and also, independently, at the Wellcome Physiological Laboratories and in Czechoslovakia, showed that the analog, in doses exerting slight or no toxic effects on the host, appreciably inhibited the growth of such tumors as lymphomas L-1210, L-4946, L-5178, and sarcoma 180,[7] adenocarcinoma 755,[2] Ehrlich ascites carcinoma,[22] and the plasma cell neoplasm 70429.[15] Since the bacterial studies had suggested that inhibition of cellular reproduction requires the formation of ribose-containing derivatives of AzU, tests were made of the urine of mice receiving AzU and the presence of material behaving in all respects as AzUR was demonstrated.[24] Further-

more, studies of sarcoma 180 cells reproducing in tissue culture showed that, although quite unaffected by AzU (in concentrations up to 5 μmoles/ml.), the growth of the neoplastic cells was inhibited by AzUR (0.01-0.02 μmole/ml.), an effect prevented by uridine but not by uracil.[19] Even greater sensitivity to AzUR was obtained with L-5178 in tissue culture; not only were these cells insensitive to free AzU, but even to its deoxyriboside[5] (the latter compound was prepared biosynthetically from AzU).[8] In these cell-systems,[10, 19] and in *S. faecalis*,[8] exposure of reproducing cells to AzUR-C^{14} led to extensive accumulation of radioactivity in the acid-soluble, but only to a very limited degree, if at all, in the nucleic acid fractions.

Since the chemical synthesis of AzUR is tedious and gives unsatisfactory yields, the conversion of AzU to AzUR was accomplished biologically using *S. faecalis*,[9] and, more recently, through the use of modifications of a ribosidation procedure involving *E. coli*, described by Skoda *et al.*[22]

The present paper describes the remarkably higher activity of AzUR, in comparison with AzU, as an inhibitor of the growth of the lymphomatous neoplasms L-1210 and L-5178, and of sarcoma 180, in mice.

MATERIALS AND METHODS

The procedures used in these experiments have been described in detail elsewhere by Mautner and Jaffe;[14] only enough information is given herein to make the data intelligible. Studies with the two lymphomas were done in AKR × DBA/2 F_1 hybrid mice of either sex (Jackson Laboratories), while studies with sarcoma 180 were done in female SWR mice (Millerton Research Farm); in both cases the animals were 6-8 weeks old, weighed 18-25 g., and were given Purina Lab Chow and water *ad libitum*. Sarcoma 180, obtained aseptically from a donor mouse, was divided into pieces of about 1 mm.[3]; one of these was implanted subcutaneously with a trocar into the right axillary region of each mouse. The lymphomas were transferred subcutaneously (right flank) as a suspension of cells; each mouse was given, within experimental error, the same number of cells ($1\text{-}3 \times 10^6$). The parent strains of both L-1210 and L-5178, obtained from Lloyd Law of the National Cancer Institute, were carried in the ascitic form in DBA/2 mice; in these experiments a special strain of the latter lymphoma termed L-5178-Y was used.*

Treatment, which began 24 hours after introduction of the neoplastic cells or tissue, was given either intraperitoneally (0.5 ml.) at intervals of 8 hours or by way of the drinking water (AzU: 0, 1.25, 2.5 and 5 mg./ml. (0, 11, 22, and 44 μmoles/ml.), respectively; AzUR: 0, 0.125, 0.25 and 0.5 mg./ml. (0, 0.5, 1.0 and 2.0 μmoles/ml.), respectively). Therapy was terminated after 6 days with the lymphomas and after 7 days with S-180. On the following day the lymphomatous mice were weighed, sacrificed, and the tumors carefully excised, weighed, and the weights compared with those

* The Y-strain of L-5178 was developed by G. A. Fischer and J. J. Jaffe from a single cell isolated *in vitro* and grown in a medium which has been described by G. A. Fischer and A. D. Welch (*Science*, 1957, *126*, 1018-1019).

of the tumors of untreated control mice; with the S-180 mice, the length of the two largest perpendicular axes of the tumors was measured *in situ* (to 0.1 mm.) and the tumor volume was estimated, using the formula for determining the volume of a prolate spheroid, and the volumes obtained were compared to those of the tumors in the untreated control mice. In Table 1 the results are presented in terms of mean values, together with the *standard error* of each mean; the effectiveness of each dosage regimen is indicated by the approximate percentage of inhibition of tumor growth.

RESULTS

The results obtained are summarized in Table 1. In addition, an experiment was carried out to test the effect of frequency of administration of AzUR on its efficacy. Earlier it had shown[7] that AzU is maximally effective against L-1210 lymphomas when given for 6 days in the drinking water or when the total daily doses (15 mg./20-g. mouse/day) were given in three portions at 8-hour intervals (83 per cent inhibition). If the same total amount was given only once daily, the inhibition obtained was 41 per cent, while administration twice daily, at intervals compatible with a normal working day, 9 a.m. and 5 p.m., gave only 50 per cent inhibition. Since other evidence indicated that AzUR is excreted even more rapidly than is AzU,* the relation between frequency of dosage of AzUR and its degree of carcinostatic effect was investigated (with L-5178-Y). It was found that insignificant inhibitions of tumor growth were obtained when the drug was given less frequently than at 8-hour intervals.

DISCUSSION

The data obtained from the comparative studies of the effect of AzUR and AzU on the growth of mouse tumors L-1210, L-5178-Y and sarcoma 180 show clearly that, on a molar basis, the carcinostatic activity of the riboside is from 10 to 20 times that of AzU.

Although the inhibitory effect of AzU on the growth of sarcoma 180 is not dramatic, the effects of AzUR, in molar ratio of 1:22 (AzUR:AzU), were clearly as great or greater than were those of the free base. Also, with L-1210 and L-5178-Y the comparisons of AzUR and AzU leave no doubt concerning the far greater potency of AzUR as an inhibitor of the very rapid growth of these lymphomas, which eventually cause a fatal leukemia. It is believed that the limited capacity of the mouse to convert AzU to

* Preliminary experiments in collaboration with L. Peters and R. Volle, Department of Pharmacology, University of Kansas, have shown that, in the hen, renal tubular secretion contributes to the excretion of AzUR but not to that AzU. Preliminary studies in man with S. C. Finch, Department of Internal Medicine, Yale University School of Medicine, also have shown that AzUR is cleared more rapidly from the blood and excreted more rapidly by the kidney than is AzU.

TABLE 1

Compound	Dosage level*	L-1210 lymphoma			L-5178-Y lymphoma			Sarcoma 180		
		Average change in body wt.	Average tumor wt.†	Approx. inhibition	Average change in body wt.	Average tumor wt.†	Approx. inhibition	Average change in body wt.	Average tumor volume‡	Approx. inhibition
By injection	*μmoles/20g./8 hr.§*	*g.*	*mg.*	*%*	*g.*	*mg.*	*%*	*g.*	*mg.*³	*%*
AzU	0	+1.0	412±26		+2.8	519±41		—2.0	1134±112	
	11	—0.8	205±14	50	+1.2	472±34	9	—3.0	1075±65	5
	22	—0.8	158±16	62	+0.8	328±26	37	—1.0	980±64	14
	44	—2.2	70±6	83	—1.2	212±16	59	—1.5	705±48	38
AzUR	0	+1.0	342±32		+2.5	646±28		0	1135±188	
	0.5	+0.5	203±13	41	+0.2	464±28	28	—1.0	768±112	32
	1.0	—0.5	174±15	49	—0.2	462±30	28	+1.0	782±106	31
	2.0	—0.5	106±3	69	—1.5	308±15	52	+0.5	694±94	39
In drinking water	*μmoles/ml.*									
AzU	0	+0.5	440±30		+2.8	580±34		—0.8	1027±106	
	11	0	236±25	46	+2.5	378±25	35	—0.2	816±106	21
	22	—0.5	194±16	56	+2.0	334±28	42	—1.2	596±47	42
	44	—0.5	103±9	77	+0.8	219±28	62	—1.5	416±33	60
AzUR	0	+1.5	222±15		+1.5	627±29		0	1135±188	
	0.5	+1.0	138±18	38	+1.2	530±47	16	—0.5	751±116	34
	1.0	—1.5	115±13	48	+0.8	303±21	52	0	469±61	59
	2.0	—2.5	73±7	67	—0.5	167±17	73	—1.0	531±79	53

* With L-5178-Y and sarcoma 180, each group contained 20 animals; with L-1210, each group contained 10 animals.

† The average weight of the mice in each group is presented, together with the standard error: $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

‡ Good agreement was obtained between calculated volumes (see text) and weights of tumors.

§ Compounds were injected intraperitoneally every 8 hours; dosage is expressed in μmoles per 20 g.-mouse per 8 hours, rather than in dosage per kg. per day, in order to facilitate comparison with the intake of compound in the drinking water, since it was found that all mice receiving drugs drank approximately 3 ml. per mouse per day.

ribose-containing derivatives, a reaction known to occur in the liver,[10] is responsible for the fact that AzU exerts a carcinostatic effect, for the tumors so far tested in tissue culture have not been affected by AzU per se, while AzUR is highly inhibitory to the growth of those lines studied.[5, 19] Since the ability of individual animals to form active derivatives of AzU may be variable, and may be influenced by dietary or many other factors, it would seem preferable now to give attention to the possible applications of AzUR as a carcinostatic agent, particularly in man. Another important reason for avoiding the use of free AzU in man is its ability to cause manifestations of central nervous system toxicity of varying degrees of severity when given repeatedly during several days (1-45 mg./kg./day), an effect which so far as is known is peculiar to the human species. First observed[24] in collaboration with D. H. Clement, this effect of AzU already has been studied extensively by others (Shnider *et al.*[21]; Wells *et al.*[20]). Earlier studies of AzU in dogs and monkeys had disclosed no effects on the central nervous system, and the anesthetic effect seen in rats and mice is obtained only with enormous dosages (3-5 grams of AzU per kg.).[24] Evidence already has been obtained to indicate that the central effects observed in man probably are not closely related to the narcotic activity of AzU observed in rodent species.

Studies of the toxicity of AzUR, given at 8-hour intervals to dogs, are now in progress. These have disclosed that the compound gradually depresses the total leucocyte count and eventually the platelet count without causing, as yet, evidence of those profound disturbances of the formation of new cells in the intestinal epithelium which have limited so greatly the use of many other carcinostatic agents. No evidence of an effect of chronically administered AzUR on the central nervous system of dogs or mice has been observed, except perhaps that the anorexia and resultant loss of weight, seen at the higher dose-levels, may be the result of a central effect of the compound. At present, it would appear that the potential usefulness of AzUR as a carcinostatic agent, particularly as an anti-leukemic agent, or as a possible inhibitor of the intracellular synthesis of various nucleic acid-containing viruses, will depend to a considerable degree on the absence, or the degree of severity, of undesirable effects on the central nervous system of man. Investigations now in progress are designed to obtain answers to this and related questions.

The rapid loss of unchanged AzUR from the blood and other tissues calls attention to the remarkable stability of this riboside and to the effect of ribosidation on the pathways of renal elimination of AzU. As is well known, the physiologically occurring ribosides are readily cleaved by nucleosidases and it is of considerable theoretical, as well as practical, interest that the

enzymatically formed riboside of AzU, so closely analogous to uridine in structure, is so very resistant to attack by catabolic enzyme systems. The finding that ribosidation of AzU results in its more rapid removal from the blood, because of active renal tubular secretion in the kidneys, is certain to lead to studies of the effect on tubular secretion of ribosidation of other bases either foreign to the body or of physiological significance. From the more practical standpoint, investigation of the mechanism of the renal tubular secretion of AzUR might suggest a means of interfering with this pathway which would permit a reduction in the rate at which the presently exceedingly precious compound is lost from the blood and other tissues.

Finally, it may be stated that, should additional studies of AzUR offer promise of chemotherapeutic utility, there is reason to believe that the biological conversion of AzU to its riboside will be a practicable procedure, the cost of which should not be a serious limitation on the continued investigation of this new agent.

SUMMARY

Azauridine, the riboside of 6-azauracil, is shown to be a potent inhibitor of the growth in mice of lymphomas L-1210 and L-5178-Y (a new strain of L-5178), and of sarcoma 180. On a molar basis, azauridine is from 10 to 20 times as active as 6-azauracil, and, in the amounts required, exerts a negligible host toxicity. The rapid elimination of azauridine in the urine necessitates frequent administration of the compound, when given parenterally, but its remarkable stability and resistance to enzymatic degradation permit azauridine to be given as effectively by mouth (in the drinking water of mice) as by frequent parenteral injection.

ACKNOWLEDGEMENTS

We are grateful for the invaluable help received from the staff of the Squibb Institute for Medical Research, in connection with the preparation of 6-azauracil and its riboside, and for the efficient technical assistance of Mrs. Maria Markiw and Miss Jean O'Loughlin.

REFERENCES

1. Barlow, R. B. and Welch, A. D.: A synthesis of "6-azauracil" (1,2,4-triazine-3,5(2H,4H)-dione, an analog of uracil. *J. Amer. chem. Soc.*, 1956, *78*, 1258-1259.
2. Bieber, S., Elion, G. B., Nathan, H. C., and Hitchings, G. H.: The inhibition of mammary adenocarcinoma 755 by analogs of uracil. *Proc. Amer. Ass. Cancer Res.*, 1957, *2*, 188.
3. Davis, R. J.: Unpublished data.
4. Falco, E. A., Pappas, E., and Hitchings, G. H.: 1,2,4-Triazine analogs of the natural pyrimidines. *J. Amer. chem. Soc.*, 1956, *78*, 1938-1941.

5. Fischer, G. A.: Unpublished data.
6. Greenbaum, S. B.: Potential metabolic antagonists of orotic acid. *J. Amer. chem. Soc.*, 1954, *76*, 6052-6054.
7. Hakala, M. T., Law, L. W., and Welch, A. D.: Inhibitory activity of 6-azauracil, 6-uracil methyl sulfone and related compounds on the growth of mouse lymphomas and Sarcoma 180. *Proc. Amer. Ass. Cancer Res.*, 1956, *2*, 113.
8. Handschumacher, R. E.: Studies of bacterial resistance to 6-azauracil and its riboside. *Biochim. biophys. Acta*, 1957, *23*, 428-430.
9. Handschumacher, R. E.: Metabolites of 6-azauracil formed by *Streptococcus faecalis*. *Fed. Proc.*, 1957, *16*, 191.
10. Handschumacher, R. E.: Unpublished data.
11. Handschumacher, R. E. and Welch, A. D.: Microbial studies of 6-azauracil, an antagonist of uracil. *Cancer Res.*, 1956, *16*, 965-969.
12. Holmes, W. L.: Studies on the mode of action of analogs of orotic acid: 6-uracil sulfonic acid, 6-uracil sulfonamide, and 6-uracil methyl sulfone. *J. biol. Chem.*, 1957, *223*, 677-686.
13. Holmes, W. L. and Welch, A. D.: Biological studies of analogs of orotic acid: 6-uracilsulfonic acid, 6-uracilsulfonamide, and 6-uracil methyl sulfone. *Cancer Res.*, 1956, *16*, 251-257.
14. Mautner, H. G. and Jaffe, J. J.: The activity of 6-selenopurine and related compounds against some experimental mouse tumors. *Cancer Res.*, 1958, *18*, in press.
15. Potter, M. and Law, L. W.: Personal communication.
16. Prusoff, W. H., Holmes, W. L., and Welch, A. D.: Biological investigations of 6-azathymine: a thymine analog. *Cancer Res.*, 1954, *14*, 570-575.
17. Prusoff, W. H. and Welch, A. D.: Studies on the mechanism of action of 6-azathymine. II. Azathymine deoxyriboside, a microbial inhibitor. *J. biol. Chem.*, 1956, *218*, 929-939.
18. Prusoff, W. H., Lajtha, L. G., and Welch, A. D.: Effect of the deoxyriboside of azathymine (azathymidine) on the biosynthesis of deoxyribonucleic acid by bone marrow and neoplastic cells *(in vitro)*. *Biochim. biophys. Acta*, 1956, *20*, 209-214.
19. Schindler, R. and Welch, A. D.: Ribosidation as a means of activating 6-azauracil as an inhibitor of cell reproduction. *Science*, 1957, *125*, 548-549.
20. Seibert, W.: Ueber den Mechanismus der reaktion von Kishner-Wolff-Staudinger. *Ber. dtsch. Chem. Ges.*, 1947, *80*, 494-502.
21. Shnider, B. I., Frei, E., Tuohy, J., Gorman, J., Freireich, E., Brindley, C. O., and Clements, J.: Toxicity studies and preliminary clinical evaluation of 1,2,4-triazine-3,5-dione (6-azauracil). *Proc. Amer. Ass. Cancer Res.*, 1957, *2*, 249.
22. Škoda, J., Hess, V. F., and Šorm, F.: Production of 6-azauracil riboside by *Eschericia coli* growing in the presence of 6-azauracil. *Coll. Czech. Chem. Commun.*, 1957, *22*, 1330-1333.
23. Šorm, F., Jakobovic, A., and Šlechta, L.: The anticancerous action of 6-azauracil (3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine). *Experientia*, 1956, *12*, 271-272.
24. Welch, A. D., Handschumacher, R. E., and Jaffe, J. J.: Observations on the pharmacology of 6-azauracil and related compounds. *Proc. Amer. Ass. Cancer Res.*, 1957, *2*, 259.
25. Welch, A. D., Handschumacher, R. E., and Jaffe, J. J.: Unpublished data.
26. Wells, C. E., Ajmone-Marsan, C., Frei, E., Tuohy, J. H., and Shnider, B. I.: Electroencephalographic and neurological changes induced in man by the administration of 1,2,4-triazine-3,5 (2H,4H)-dione (6-azauracil). *Electroenceph. and Clin. Neurophysiol.*, 1957, *9*, 325-332.

GILBERT H. GLASER*

Section of Neurology, Department of Internal Medicine, Yale University School of Medicine, and the Grace-New Haven Community Hospital

VISCERAL MANIFESTATIONS OF EPILEPSY

The generalized convulsion or grand mal attack of epilepsy usually presents such a striking picture of somatic motor phenomena that signs and symptoms involving visceral and other autonomic functions, while present, are overshadowed. Within the grand mal seizure there usually occur cardio-respiratory, gastro-intestinal, urinary, and other autonomic symptoms. Prior to a grand mal seizure there may be a visceral aura. This is often an epigastric or abdominal sensation and may occur in as often as 20 per cent of the patients. During a grand mal seizure patients become cyanotic, have disordered respirations, tachycardia, occasional vomiting, involuntary defecation and urination, sweating, increase in blood pressure and pupillary changes. Some of these symptoms may persist after the main portion of the seizure, and there may be a fever, especially in children.

Aside from these manifestations, occurring in some relation to a grand mal seizure, there are many instances of visceral phenomena representing in themselves the major portion of a seizure state. These often occur in patients with seizures in the psychomotor group. Psychomotor seizures are characterized usually by auras of anxiety and visceral symptoms followed by alterations in consciousness associated with many varied complex feeling and thinking states and automatic somatic and autonomic motor behavior. These phenomena are associated with at least a partial amnesia. In many instances a sequence of motor events may occur, evolving in brief phases. First, there may be arrest or suspension of activity with perhaps a simple nonrepetitive movement. This is followed by repetitive, stereotyped automatisms with little variation from seizure to seizure such as swallowing, sucking or chewing, and movements of the arms and legs. The third phase consists of automatic acts of varying complexity often associated with visceral symptoms. These automatic acts are semipurposeful, usually inappropriate, bizarre, involving an interplay with the environment and determined in part by psychological factors. The activities in this latter phase often may appear to merge into normal behavior. This type of seizure complex

* Associate Professor of Neurology and Chief, Section of Neurology.
Received for publication October 9, 1957.

represents a major example of partial epilepsy wherein certain discharges occur involving focal areas of the cortex or subcortex of the brain (especially the temporal-limbic system and its connections) and there is only a partial disturbance of consciousness. In many instances the visceral manifestations so dominate the picture that the patient may have as a presenting complaint symptoms involving gastro-intestinal, genito-urinary or cardio-respiratory systems. Often, these symptoms are diagnosed as due to localized visceral disease rather than originating as a partial seizure discharge, and in addition the common association of these symptoms with a picture of bizarre behavior or mental activity may lead to a diagnosis of a psychiatric disorder.

It is the purpose of this paper to present certain selected cases illustrating the varied visceral manifestations which may occur as the result of an epileptic discharge, to describe the associated electroencephalographic patterns, and to indicate how these manifestations fit into different types of seizure discharges.

MATERIALS AND METHODS

The cases have been selected from the Seizure Clinic at the Yale-New Haven Medical Center. All have had thorough medical and neurological evaluations, including apppropriate x-ray studies and serial electroencephalograms, the latter often during sleep and photic activation. Nasopharyngeal or basal electrodes were particularly helpful in delineating temporal lobe foci in the adult patients. No patient had a known specific structural or metabolic lesion of the nervous system. Most of the cases have had psychological and psychiatric evaluations, and in many instances, especially with the adult patients, sodium amytal interviews were conducted in order to obtain further detailed recollections concerning experiences during seizures.

Case I: M.V. Age 18. This boy has been followed since the age of four. At the age of two and a half he began to have spells of sleepiness, frontal headaches, retching and vomiting, and fever to 102 to 104° F. Between the ages of four and five he had many electroencephalograms, a ventriculogram, and a pneumoencephalogram, all of which were normal. He would have as many as three of these spells per day each lasting up to one hour. In 1946 at the age of eight, his EEG showed the first abnormalities with spikes coming from the right temporal region (Fig. 1). As time went on, the frequency of the seizures decreased spontaneously and there was never any evidence of definite response to anticonvulsant medication such as phenobarbital and dilantin. Psychological factors were reviewed in great detail and did not lead to any significant insight into the symptoms. At the age of 15 he was given dexadrine, his attacks finally were eliminated, and the EEG became normal.

Case II: J.V. Age 9. This patient is a sister of the above young man. She began to have seizures at the age of fifteen months following an episode of chickenpox. Her spells were originally characterized by generalized rigidity, putting the left arm above the head, crying, and some alteration of consciousness. Her hands and feet were often a bluish-black color during the spell. Aside from several of these episodes, she had one

generalized convulsion with total loss of consciousness. Her EEG was normal and she was treated with phenobarbital which controlled the seizures. Six years later she began to have episodes associated with generalized headaches, nausea, pain in the stomach, and a fever of 102 to 104° F. During some of these spells she would have either flushing of the face or pallor. She usually remained drowsy for almost an hour during these episodes, and there was no total loss of consciousness.

Case III: C.N. Age 8. This child has a history of intractable paroxysmal abdominal pain followed by psychomotor seizures since the age of one and a half. The child was jaundiced at birth due to erythroblastosis and had jerking movements of the extrem-

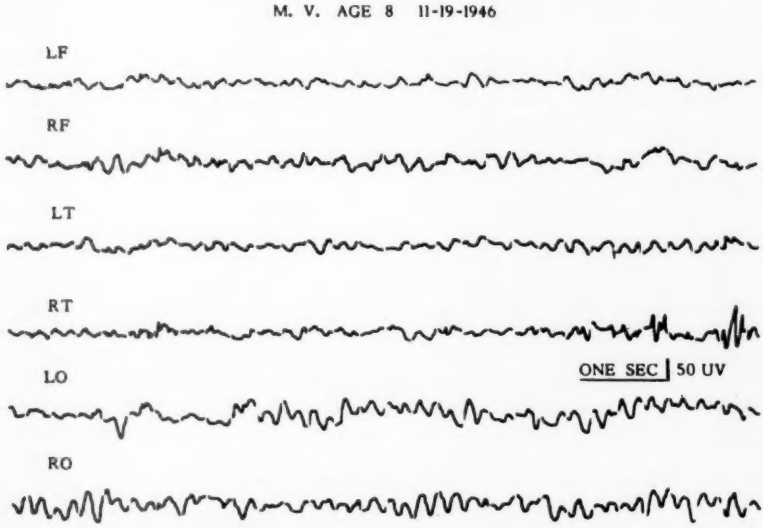


FIG. 1. Electroencephalogram of Case 1. Focal spike discharge from the right temporal region at age eight, four years after onset of seizures.

ities and lethargy for three days. At the age of 15 months she had a febrile convulsion associated with probable exanthem subitum. Shortly after this she began to have seizures which have remained about the same in pattern. Often she would have five episodes a day but has had clear periods of two to four weeks. She has an aura of severe abdominal pain lasting for somewhat less than a minute and she clutches her abdomen. There is momentary staring and alteration of awareness. She often places one hand, usually the left, to her face, and there is some cyanosis and occasional sucking movements of the lips. She has lost bladder control on several occasions. There is at times incoherent mumbling but no definite speech. She does hear during the spell and partial contact can be made. The attacks last in duration up to about five minutes, and the termination is often abrupt. Occasionally, she only has episodes of severe abdominal pain or discomfort without any of the symptoms. Her electroencephalograms have shown only occasional suggestive spike formations, often associ-

ated with slow waves, from the temporal lobes especially on the left side. In between her seizures she has a great deal of behavioral difficulty of an impulsive nature. Her psychological tests showed a borderline intelligence and evidence of central nervous system dysfunction. She has not responded well to any anticonvulsant medication. At one time it was determined that during her attacks her stomach "knotted up." She was given tincture of belladonna and other similar antispasmodic agents to maximum dosages without any significant effect.

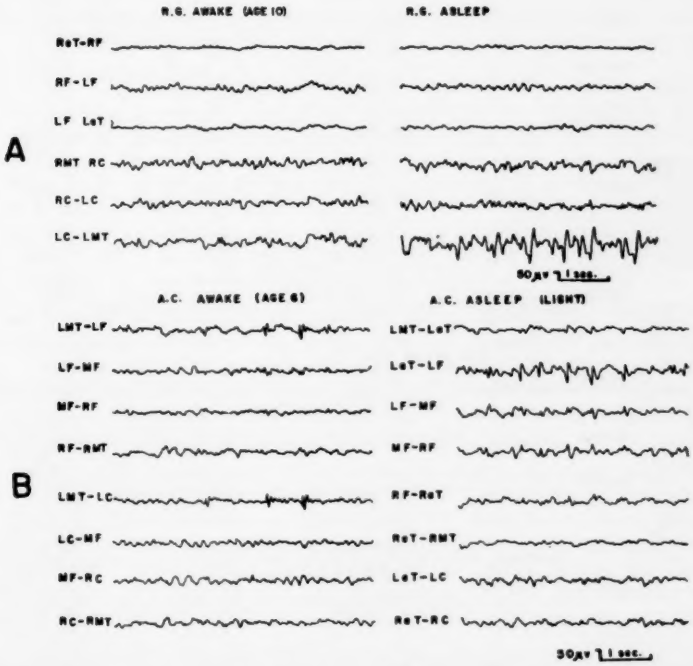


FIG. 2. Electroencephalograms of patients with psychomotor seizures and visceral manifestations: temporal lobe foci.[5]

A. Left middle temporal slow spike or sharp wave focus brought out by sleep (Case IV).

B. Left middle temporal spike focus during waking state; prominent left anterior temporal slow spike or sharp wave focus during sleep (Case V).

Case IV: R.G. This ten-year-old boy had a history of febrile convulsions at the ages of eight months and two years. He began to have psychomotor seizures at the age of nine characterized by an aura of a peculiar taste in the mouth followed by vomiting and a speech disturbance. There were aimless movements of the right arm and leg during the seizure. His EEG is characterized by high voltage slow spikes or sharp waves from the left middle temporal region (Fig. 2a).

Case V: A.C. A girl aged six had a history of two months of seizures characterized first by staring ahead and then vomiting. This was followed by her becoming limp in all extremities and then she was dazed and confused for several minutes. Her EEG during the waking state showed occasional intermittent spikes from the left middle temporal region, but during sleep the spikes increased in amplitude and tended to predominate from the left anterior temporal and frontal areas (Fig. 2b).

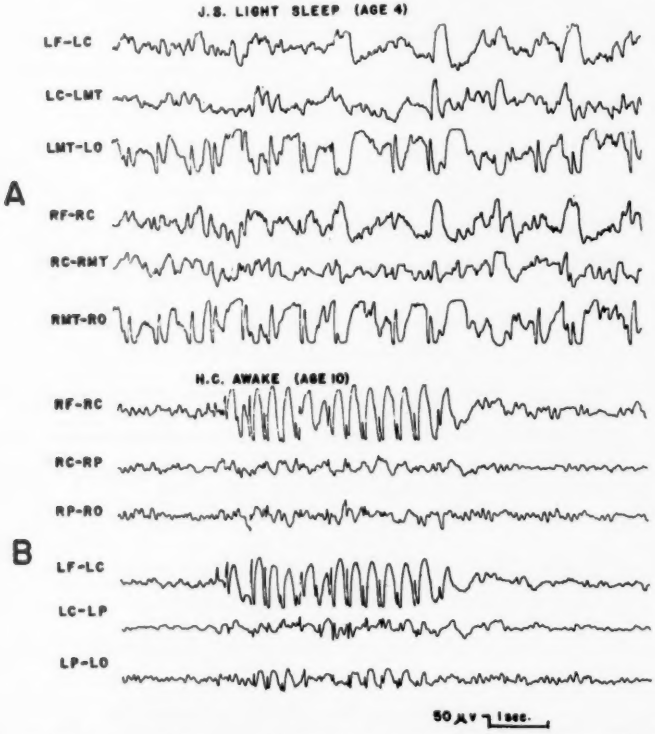


FIG. 3. Electroencephalograms of patients with psychomotor seizures and visceral manifestations: bilateral spike-wave discharges.[5]

A. Bilateral 1½ to 2 c/sec. spike-wave discharges mainly from the temporal and occipital regions during light sleep (Case VI).

B. Bilateral synchronous 3 c/sec. spike-wave discharges appearing symmetrically, mainly from the frontal and central regions (Case VII).

Case VI: J.S. A boy aged four had a one-and-one-half-year history of spells usually beginning with a headache. During the spell he would sit up, if he were lying down, or, if he were standing, he would become limp and fall to his knees. This would be followed by retching or vomiting and then gagging, spitting, or gurgling movements or sounds for about 15 minutes. His EEG showed bilateral 1½-2 cycle per second

spike and wave discharges appearing mainly from the temporal and occipital regions (Fig. 3a).

Case VII: H.C. A ten-year-old girl had a history of two years of seizures characterized by a dazed look following which the child often would spit, retch a few times, and wander around the room in a confused fashion. During the seizure she would have a blank stare and appear to be dazed, disoriented, and out of contact for several minutes. Following this, she complained of severe midabdominal pain. She had several grand mal seizures at the age of six. Her EEG contained bilaterally synchronous high voltage 3 cycle per second spike and wave discharges occurring primarily from the frontal and central regions. There was never any history of petit mal seizures at any time (Fig. 3b).

Of a series of 25 children between the ages of 1 and 16 with psychomotor seizures[4] 14 had epigastric sensations as an aura or a sensation just in the beginning of the seizure, often associated with nausea and vomiting. Four of these children had severe abdominal pain. One child experienced severe thirst in association with the epigastric sensation. Pallor or flushing of the face occurred in nine of the children in an early phase of the attack.

The following adult patients with psychomotor seizures have varied visceral manifestations associated usually, but not always, with either unilateral or bilateral temporal lobe abnormalities in the electroencephalograms.

Case VIII: J.L. This 24-year-old man has seizures characterized at onset by a choking, gagging sensation. He has stated: "It is hard to breathe, I gasp and choke, it's a choking sensation in my throat. I also have a funny feeling in my stomach." The patient also has a peculiar taste and smell in association with the other feelings. His EEG contains bitemporal random theta activity, unaltered during sleep and not determined more focally with nasopharyngeal electrodes.

Case IX: F.F. The seizures of this 39-year-old woman are characterized by a peculiar feeling in the epigastrium going to the head and accompanied by nausea, gagging, retching, and vomiting of watery material. She is out of contact with the environment, mumbles, and makes various automatic movements with her hands. Under sodium amytal she described that the feeling in her stomach was burning and went up and down from her head to her stomach and vice versa. She feels that this was a little different from ordinary nausea. She does not recall having a sweaty sensation in association with this. After each seizure she has a "terrible taste" in her mouth. A focus of spike activity from the right anterior temporal and basal regions is present in her EEG, especially during sleep.

Case X: M.H. This 28-year-old woman has psychomotor seizures often preceded by a peculiar shivering and cold feeling going up and down her body associated with nausea and mild abdominal pain. Her EEG contains spikes appearing from the right anterior, posterior temporal, and basal regions.

Case XI: L.R. This 33-year-old woman has severe pains in her stomach at the onset of seizures prior to the occurrence of visual hallucinations and automatic movements. She occasionally has urinary incontinence. Her EEG contains only moderate amounts of random theta activity from all areas, especially the parietal.

Case XII: J.B. This 32-year-old man has psychomotor seizures which were precipitated in the laboratory often by light stimulation. He has severe gastric distress prior

to the onset of his seizures characterized by dizziness, vertigo, involuntary movements and generalized automatisms. Only diffuse random theta activity is present in his EEG, with no further changes brought out during sleep or with basal electrodes.

Case XIII: E.M. A 38-year-old woman has psychomotor seizures preceded by nausea, an epigastric discomfort traveling up to the head and a feeling of "rolling" in the stomach. Her EGG is characterized by random theta activity, especially bitemporal.

Case XIV: R.A. This 32-year-old man describes the following sensations prior to all of his seizures: "The abdominal sensation occurs almost all the time, I believe. It is a sort of bursting of an inner wall. Perhaps the stomach or part of the intestines, as though they were actually splitting open. It is quite painful. There is also a feeling of

TABLE 1. AUTONOMIC-VISCERAL MANIFESTATIONS OF EPILEPSY

Gastro-intestinal	*Cardiorespiratory*
Nausea, vomiting	Asphyxia, choking, cyanosis
Salivation	Thoracic discomfort
Epigastric-abdominal discomfort	Hyperventilation
Borborygmi-belching	Hiccoughing
Involuntary defecation	Tachycardia, palpitation
	Heart block
Miscellaneous Autonomic	
Rising sensations	Paroxysmal hypertension
Sweating	Blanching or flushing
Pilomotor	Erythema, urticaria
Fever	Pupillary changes
Shivering, chill	Genital (priapism, orgasm)
Thirst	Involuntary urination

fright when this bursting sensation is at its height. After this happens, repetitive thoughts and talking occur through my mind and I see somebody chasing me." The patient then has automatisms which he does not remember. His EEG shows a focus of spikes and sharp waves from the left anterior and posterior temporal region, especially during sleep.

Case XV: R.S. This 21-year-old male college student has seizures preceded by epigastric discomfort associated with a great deal of fear. Tense feelings travel "up to my head." He has a desire to lie down and try to drive out the feelings. After these sensations he has automatic movements in a dazed, dissociated state of which he has no recollection. A right posterior temporal theta wave focus has been present in his EEG on some but not all occasions.

DISCUSSION

Various visceral symptoms as manifestations of epilepsy have been recognized for a long time.[19] A summary listing of these is shown in Table 1. One of the earliest instances described is by Morgagni,[18] in 1761, of a priest, aged

68, who had recurrent seizures beginning with a pain in the right hypochondrium followed by a sensation "of a kind of smoke ascending up to the head," then nausea and vomiting. Morgagni further found that these attacks could be suppressed by having the patient smell spirits of ammonia. These symptoms were followed by a generalized convulsion associated with difficulty in breathing, involuntary urination, and a "rarity of the pulse." Slowing of the pulse has been reported occasionally in association with epileptic seizures. Almost two hundred years later, in 1954, a case was reported[28] of a 71-year-old woman with periodic heart block associated with loss of consciousness. This patient's EEG contained abnormal spike discharges from the right anterior temporal region.

During the 19th century, visceral symptoms associated with epilepsy were described in detail by Trousseau[27] who discussed these phenomena in differential diagnosis from cardiac and abdominal disease. Later Jackson,[11] Gowers,[6, 7] and Wilson[28] presented fuller descriptions and discussed visceral, especially epigastric, aurae. During the past 20 years a greater understanding of these phenomena has developed from the work of Penfield and Jasper[3] and Gastaut.[8] Association of isolated paroxysmal abdominal pain with abnormal electroencephalograms has been discussed in terms of abdominal epilepsy by Moore,[17] Hoefer,[9] and Livingston.[13] One of Hoefer's patients had transient intussusception during an attack. In the majority of these instances, however, the visceral manifestations have been associated with other, more clear-cut, evidence of the existence of a seizure state, especially of the psychomotor type. There is also the matter of abnormal electroencephalograms in over 90 per cent of the cases, especially those with unilateral or bilateral temporal lobe discharges in adults, and these abnormalities, plus bilaterally diffuse spike and wave discharges, in children.[5] The diagnosis of the visceral manifestation as being epileptic, therefore, is inferential except when one can observe paroxysmal electroencephalographic abnormalities appearing at the same time as the visceral phenomenon, an unusual, relatively rare coincidence in the clinical evaluation of these patients. Cases have been described with episodic abdominal pain associated with synchronous high voltage 2-3 cycle per second waves during attacks and milder abnormalities in the free periods.[12, 20]

Several of the adult patients in our series described an inordinate experience of fear, especially in association with a preceding gastric sensation as an aura. This relatively common sequence recently has been commented upon by Stevens[26] in her review of the possible "march" of temporal lobe epilepsy, further emphasizing the strong interrelationship between visceral sensations and emotional experiences.

There has been a great deal of neurophysiological investigation concerning the eliciting of visceral phenomena in man and experimental animals by stimulation of various parts of the brain. Earlier work concerning the autonomic representation in the cerebral cortex indicated that gastro-intestinal activity could be influenced by stimulation of the motor and premotor areas. In addition, the localization of autonomic phenomenon in the hypothalamus and adjacent regions of the diencephalon has been well known

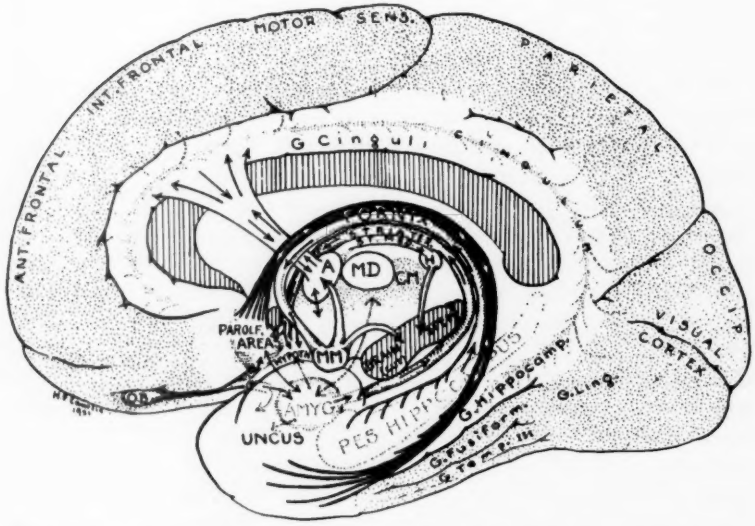


FIG. 4. The limbic system and its principle subcortical connections viewed from the mesial surface of one hemisphere. The connections of the brain stem reticular formation have been omitted. (From: Penfield, W. and Jasper, H. H.[22])

since the work of Hess,[8] and Ranson and Magoun.[25] In clinical investigations, Cushing[3] described gastric and duodenal ulcers in patients with tumors of the diencephalon. More recently, however, the kinds of visceral phenomena occurring in seizure states have been elicited by stimulation of that region of the brain now included in the limbic system (Fig. 4). This represents the old rhinencephalon mostly within the temporal lobe and the perifalciform areas. Stimulation of these areas in animals and man[1, 12, 14, 16, 24] causes changes in blood pressure, respiration, and gastro-intestinal motility associated with alterations in pupillary size, piloerection, sweating, and salivation. Newer confirming data concerned with visceral functions of the

brain have been recently reviewed.[15] Observations in man have shown that changes in gastric motility and upper abdominal pain may be evoked by stimulation especially of the insula.[21] Stimulation of the uncus has induced apnea with marked clouding of consciousness.[24] It is of interest that in 1899 Jackson[10] reported a case of paroxysmal asphyxia associated with cyanosis and a dreamy state occurring in a patient without any convulsive movements. However, in instances of paroxysmal visceral pain assumed to be on the basis of a seizure mechanism the experience of the pain by the patient may exist without any abnormal visceral activity, such as disordered intestinal or gastric motility. Thus, this type of sensory seizure may be a primary perceptual disorder of central visceral sensory centers, or a secondary experience derived from peripheral autonomic dysfunction centrally induced.

The response of these manifestations to anticonvulsant therapy is extremely variable. If the patient has grand mal seizures in association with his visceral phenomena, there is usually a response to anticonvulsant medication and both states tend to be helped. This is less true with psychomotor seizures. The various paroxysmal visceral manifestations existing alone often are refractory to anticonvulsant therapy. In instances of excessive gastro-intestinal activity and paroxysmal abdominal pain, there have been treatment attempts using antispasmodics, especially of the atropine group and related synthetics. Usually, no significant results have been obtained.

SUMMARY AND CONCLUSIONS

Varied visceral manifestations involving gastro-intestinal, cardiorespiratory, and other autonomic functions may occur with major or minor seizure states or independently as paroxysmal phenomena. Association with psychomotor seizures of temporal lobe and subcortical origin is most common along with characteristic electroencephalographic correlations. The visceral disorder, however, may be experienced either as a primary perceptual disturbance of central visceral sensory centers or as a secondary experience derived from a centrally induced dysfunction of peripheral autonomic structures. The anatomic circuits involve mainly the limbic system, motor and premotor cortex, and the hypothalamus. Therapeutic response to anticonvulsant medication or drugs affecting the autonomic nervous system is inconsistent.

ACKNOWLEDGEMENTS

The author is grateful to the *Journal of Electroencephalography and Clinical Neurophysiology* for permission to reproduce Figures 2 and 3, and to Little, Brown and Company for permission to reproduce Figure 4.

REFERENCES

1. Anand, B. K. and Dua, S.: Electrical stimulation of the limbic system of brain (visceral brain) in the waking animal. *Indian J. med. Res. Mem.*, 1956, *44*, 107-119.
2. Cushing, H.: Peptic ulcers and the interbrain. *Surg. Gynec. Obstet.*, 1932, *55*, 1-34.
3. Gastaut, H.: *The epilepsies. Electroclinical correlations.* Springfield, C. C Thomas, 1954, pp. 23-24, 57.
4. Glaser, G. H. and Dixon, M. S.: Psychomotor seizures in childhood. A clinical study. *Neurology*, 1956, *6*, 646-655.
5. Glaser, G. H. and Golub, L. M.: The electroencephalogram of psychomotor seizures in childhood. *EEG clin. Neurophysiol.*, 1955, *7*, 329-340.
6. Gowers, W. R.: *Epilepsy and other chronic convulsive disorders: Their causes, symptoms and treatment.* 2d Ed. London, J. and A. Churchill, 1901, pp. 37-83, 106-107, 134-147.
7. Gowers, W. R.: *The borderland of epilepsy.* London, J. and A. Churchill, 1907, pp. 18-39.
8. Hess, W. R.: *The diencephalon. Autonomic and extrapyramidal functions.* New York, Grune and Stratton, 1954, pp. 12-24, 40-45, 51, 63.
9. Hoefer, P. F. A., Cohen, S. M., and Greeley, D. McL.: Paroxysmal abdominal pain. A form of epilepsy in children. *J. Amer. med. Ass.*, 1951, *147*, 1-6.
10. Jackson, J. H.: Neurological fragments No. XVIII. On asphyxia in slight epileptic paroxysms—on the symptomatology of slight epileptic fits supposed to depend on discharge-lesions of the uncinate gyrus. *Lancet*, 1899, *1*, 79-80.
11. Jackson, J. H.: *Selected writings of John Hughlings Jackson.* J. Taylor, Ed. Vol. I. *On epilepsy and epileptiform convulsions.* London, Hodder and Stoughton, Ltd., 1931, pp. 118, 276-307, 458-473.
12. Kaada, B. R.: Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of "rhinencephalic" and other structures in primates, cat and dog. *Acta physiol. scand. (Supp. 83.)*, 1951, *24*, 1-285.
13. Livingston, S.: Abdominal pain as a manifestation of epilepsy (abdominal epilepsy) in children. *J. Pediat.*, 1951, *38*, 687-695.
14. MacLean, P. D.: The limbic system and its hippocampal formation: studies in animals and their possible application to man. *J. Neurosurg.*, 1954, *11*, 29-44.
15. MacLean, P. D. and Delgado, J. M. R.: Electrical and chemical stimulation of the fronto-temporal portion of the limbic system in the waking animal. *EEG clin. Neurophysiol.*, 1953, *5*, 91-100.
17. Moore, M. T.: Abdominal epilepsy. *Rev. Gastroent.*, 1948, *15*, 381-395.
18. Morgagni, G. B.: *The seats and causes of diseases investigated by anatomy; in five books, containing a great variety of dissections, with remarks.* Translated by B. Alexander. London, A. Milla and T. Cadell, 1769, Vol. I, pp. 192-195.
19. Mulder, D. W., Daly, D., and Bailey, A. A.: Visceral epilepsy. *Arch. int. Med.*, 1954, *93*, 481-493.
20. O'Brien, J. L. and Goldensohn, E. S.: Paroxysmal abdominal pain as a manifestation of epilepsy. *Neurology*, 1957, *7*, 549-554.
21. Penfield, E. and Faulk, M. E.: The insula: further observations on its function. *Brain*, 1955, *78*, 445-470.
22. Penfield, W. and Jasper, H. H.: *Epilepsy and the functional localization of the human brain.* Boston, Little, Brown and Company, 1954, pp. 26, 106-108, 177-179, 412-437.
23. Phizackerly, P. J. R., Poole, E. W., and Whitty, C. W. M.: Sino-auricular heart block as an epileptic manifestation. *Epilepsia (Boston)*, 1954, *3*, 89-91.
24. Pool, J. L.: Visceral brain of man. *J. Neurosurg.*, 1954, *11*, 45-63.
25. Ranson, S. W. and Magoun, H. W.: The hypothalamus. *Ergebn. Physiol.*, 1939, *41*, 56-163.
26. Stevens, J. R.: The "march" of temporal lobe epilepsy. *Arch. Neurol. Psychiat. (Chicago)*, 1957, *77*, 227-236.
27. Trousseau, A.: *Lectures on clinical medicine, delivered at the Hôtel Dieu, Paris.* Translated and edited by P. V. Bazire. London, Robert Hardwicke, 1867, Vol. I, pp. 39-104.
28. Wilson, S. A. K.: Epileptic variants. *J. Neurol. Psychopath.*, 1928, *8*, 223-240.

GUSTAF E. LINDSKOG*

Department of Surgery, Yale University School of Medicine

A HISTORY OF PULMONARY RESECTION**

Except for the heart itself, the lung is the last of the major viscera to yield to the surgeon's scalpel. As late as 1896, Stephen Paget,[84] a distinguished, although apparently somewhat pessimistic and pragmatic British surgeon, wrote concerning malignant tumors of the chest:

"... We have to consider only what hope can be offered to these cases by surgery. The most that can be done, and, so far as we can see, that ever will be done is to withdraw the effusion. The one or two procedures of a more serious nature that have been recorded have been rather matters of chance than formal operations. . . ."

When one recalls that the first successful *subtotal* pneumonectomy for bronchiectasis was accomplished in 1931, and the first for cancer in 1933, it might appear that the technical trials of pulmonary resection have encompassed only the brief span of two recent decades. On the contrary, in addition to the necessary basic developments in hemostasis, in asepsis, and in anesthesia (especially the technique of positive pressure endotracheal insufflation) the struggle to develop a safe and effective method of removing all or portions of one lung has continued over a period of not less than five centuries.

It is not surprising to learn that Galen,[37] that ancient writer with catholic interests, experimented with the problem of ventilatory dynamics. This native of Pergamon in Asia Minor who studied anatomy at Alexandria and eventually settled in Rome to become its leading practitioner of the medical arts early in the second century A.D. was not only an anatomist and physician but the first recognized experimental physiologist. His great curiosity and imagination led him to consider the problems of respiratory mechanics. So we read of an ingenious experiment in *De anatomicis administrationibus* (Book VIII, Chapter 10, translation of Charles Singer).

> There is another experiment which is thought to show that some of the air filters through to the pleural cavity from the lungs. Prepare in advance a bladder with a mouth of suitable size. Then cut the skin over the ribs in a circle, so that the area of

---

* William H. Carmalt Professor of Surgery; Surgeon-in-Chief, Grace-New Haven Community Hospital University Service.

** The Second Annual Samuel Clark Harvey Lecture, February 4, 1957.

the cut is the same size as the mouth of the bladder. Then excise the rib. . . . Next sew the bladder to the lip of the wound, putting the mouth of the bladder underneath all around, so that the skin is outside. Now seal the holes made in it with a needle and thread and some plastic substance like . . . wax. There is now no perceptible gap between thread and skin, for what escapes the eye is safely closed with the wax preparation, so that no air can pass in from the surrounding atmosphere, nor from the inside out. Now perforate the bladder at its end and insert through the hole a scalpel with a round handle, so that, when a suture is put round the bladder outside, its membrane is tied round the handle of the scalpel so that nothing can escape between it and the bladder and the handle. . . . Then incise the pleura with the scalpel and observe how through the incision air percolates from the thorax into the bladder during exhalation. During inhalation, when the thorax is distended you will see this air once more drawn into the thorax through the incision, and then again passing into the bladder as the animal exhales and from it again entering the thorax. . . .

This is an early example, but unfortunately not the last, of a well-designed experiment gone wrong because of a technical fault.

From the time of Galen to the middle of the nineteenth century the only recorded examples of pulmonary resection were those which may be considered in one respect or another as fortuitous. They concerned wounds of the chest wall which permitted parts of the lung to prolapse, or spontaneous and post-traumatic suppuration of the chest wall and pleura which bared the underlying lung so that infected and necrotic portions could be removed without invasion of the free pleural space.

In the traumatic category is an early medieval case recounted by Roland (Capelluti) in his *Chirurgia.* This surgeon of Parma was called to treat a Bolognese youth who presented himself on the sixth day after a grievous thoracic wound with an extruding gangrenous lobe. The necrotic tissue was excised and the patient survived, regaining sufficient health to accompany his master on a subsequent expedition to the Holy Lands. According to Castiglioni,[7] Theodoric (1205-1298) mentioned this same case in a manuscript now in the library of St. Mark's (Venice) and stated that the actual operation was not performed by Roland but by Hugh de Lucca (d. 1252) with Roland acting as his assistant. This contention was possibly actuated by family loyalties since Fr. Theodoric was a Borgognoni and reputedly the son of Hugh de Lucca.

Tulpius[4] tells of a case treated prior to 1674. A brawling fellow sustained a deep wound below the left nipple, but was too drunk to heed it or call for help. The next morning a portion of lung about three fingers in breadth was noted to be extruding. With the unprotected lung still hanging out, the wounded man undertook a two-day journey afoot to Amsterdam. There Tulpius cut off the prolapsing segment. He emphasized that he weighed it on a scale. The fragment weighed three ounces—in his words "Grande

mehercules pondus, in viscere tam raro ac levi (an enormous weight for an organ so tenuous and light)." The patient mended quickly, save for an occasional light cough. Sad to relate, a continuing propensity for revelry led to his downfall and involuntary appearance in the anatomical theater six years later.

Tulpius was Nicholas Tulp (1593-1674), professor of anatomy at Amsterdam. He was the principal subject of Rembrandt's famous painting "The Lesson in Anatomy of Dr. Tulp," one of the highest priced paintings of all times, now in the Hague Museum.

A similar case was presented by Fabricius Haldanus[35] in his *Opera omnia,* a case communicated by Abelus Roscius in 1606. The identical patient is mentioned by the German surgical master, Laurence Heister[36] in a footnote in his *Chirurgie* (1724). Heister gave advice on dealing with chest wounds when the lung was prolapsed. His comments indicate that he appreciated the need of keeping the pleural cavity air-tight and therefore the importance of delaying amputation of a strangulated lobe until adherence of the lung to the wound edges was assured. These are principles so basic to pulmonary surgery in its formative period (and even until quite recent times) that it seems appropriate to render a translation of his remarks.

> When an injured portion of lung presents in the external wound (as Fontanus, Tulpius and Ruysch have observed) one should not, if it is firmly attached, attempt to thrust it back into the thoracic cavity, because it might bleed. One should seek to favor healing with applications of balsam, and plasters and by rest for the patient maintained until the lung can develop adhesions without [further] injury and the wound finally heals.
>
> When, however, the injured portion of lung hangs entirely outside the wound, one should apply a soft dressing over it, and using a stout suture firmly approximate it to the wound. One end of the thread about a foot long should be left hanging so that after separation of the lung fragment it can be withdrawn from the wound. Distal to the ligature one cuts away the extruded fragment, pressing back the remaining lung gently into the chest cavity with a finger, again letting the ligature hang from the wound and applying a wick to the wound to keep it open until the thread separates from the lung after several days. The chest [wound] is thoroughly cleansed, to which end one may use the same injections we have learned [to employ] in abdominal wounds. By these means one will prevent further bleeding from the lung and all the other dreaded complications. . . .

As Heister recalled, another case was reported by Frederik Ruysch (1638-1731). Ruysch was an anatomist, and a student of Swammerdam who taught him the technique of vascular injection using fats of low melting point and waxes. Ruysch's anatomical preparations became so famous that one time he was visited by no less a personage than Peter the Great who purchased materials to be used in the Anatomical Institute at

Saint Petersburg (now Leningrad). Quoting from the French translation of Ruysch's original Latin[40]:

. . . An apprentice sailor, having been wounded in the lower anterior chest, went to a surgeon who noted that a small part of the lung was extruding from the wound. Mistaking it for a bit of the omentum, he ligated straightway all of the presenting portion, and then called me into consultation. He was extremely surprised when I made it known that the wound penetrated, not the abdominal, but the thoracic cavity, and that the presenting part which he had ligated was lung. Things being as they were, I advised him to leave the wound alone, to dress it once a day with a digestive and a protective plaster until the ligated part was entirely necrotic and separated by itself. I hoped thereby that the portion of lung proximal to the ligature would become adherent to the wound. Happily this [stratagem] was so successful that the patient was perfectly cured in a short time."

William Forde, an obscure British military surgeon on duty with the 72nd Highlanders in South Africa reported another example in 1836.[16] This time the victim was a Fingo native whose chest was completely transfixed by the azigai [*sic*] of a hostile Kaffir. When seen by Assistant Surgeon Forde the next day a portion of lung about five inches in length was protruding through the narrow wound. After allowing the lung to adhere for three days, it was excised with a scissors and the wound was healed at the expiration of a fortnight.

About a century after Heister, Conrad J. M. Langenbeck,[20] professor of anatomy and surgery at Göttingen, gave a brief exposition on the management of chest wounds in his *Bibliothek für die Chirurgie.*

"Deep lung wounds caused by sharp, small instruments often heal in a few days, but after several weeks cause a new abscess [to develop]. Often a portion of lung prolapses from the wound, and because of constriction, and irritation of the atmosphere, becomes brown, blue and dried out. These must without hesitation be replaced, unless obviously gangrenous—black and necrotic. . . ." Langenbeck had nothing further to say about the extirpation of the gangrenous cases. Perhaps he had learned from bitter experience to leave them alone!

In the second category, that of chest wall destruction with exposure, and even loss, of pulmonary substance by a suppurative process, there is a very graphic account by William Harvey[47] in *Exercitationes de generatione animalum* (London, 1651). Although not actually an example of pulmonary resection, a portion of his observations follows:

A young nobleman, eldest son of the Viscount Montgomery, when a child had a severe fall, attended with fracture of the ribs of the left side. The consequence of this was a suppurating abscess, which went on discharging abundantly for a long time, from an immense gap in his side. . . . His serene Majesty King Charles [the ill-fated Charles the First of England] straightway sent me to wait on the young man, that I might

ascertain the true state of the case. And what did I find? A young man, well grown, of good complexion, and apparently possessed of an excellent constitution, so that I thought the whole story must be a fable. Having saluted him according to custom, and informed him of the king's expressed desire that I should wait upon him, he immediately showed me everything, and laid open his left side for my inspection, by removing a plate which he wore there by way of defence against accidental blows and other external injuries. I found a large open space in the chest, into which I could readily introduce three of my fingers and my thumb; which done, I straightway perceived a certain protuberant fleshy part, affected with an alternating extrusive and intrusive movement. . . . Amazed with the novelty of such a state, I examined everything again and again, and when I had satisfied myself, I saw that it was a case of old and extensive ulcer, . . . brought by a miracle to a kind of cure, the interior being invested with a membrane, and the edges pr tected with a tough skin. But the fleshy part, (which I at first sight took for a mass of granulations, and others had always regarded as a portion of lung) from its pulsating motions and the rhythm they observed with the pulse . . . as well as from their discordance with the respiratory movements, I saw was no portion of the lung that I was handling, but the apex of the heart! covered over with a layer of fungous flesh by way of external defence, as commonly happens in old foul ulcers. The servant of this young man was in the habit daily of cleansing the cavity from its accumulated sordes by means of injections of tepid water; after which the plate was applied, and, with this in its place, the young man felt adequate to any exercise or expedition, and, in short, he led a pleasant life in perfect safety. . . .

Those readers who are old enough to remember the appearances of the chest wall in patients after cautery pneumonotomy or the radical Schede type of thoracoplasty will recognize their similarity to the description of the young Montgomery only too well!

A later observation in this same category took place during the post-Revolutionary period in the state of Georgia. This is the much-quoted case described by Dr. Milton Antony[1] in the *Philadelphia Medical Journal* of 1823. Dr. Antony presented such a beautifully literate and detailed case report, and so candid an exposé of the tribulations endured by a brave and resourceful surgeon treading on "terra incognita" that it is worthwhile to quote directly from the report in abbreviated fashion:

CASES

Art. XII. Case of extensive caries of the fifth and sixth Ribs, and disorganization of the greater part of the right lobe of the Lungs, with a description of the operation for the same, etc.

Communicated by Milton Antony, M.D.

On the 3d of March 1821, I made my first visit to Elmon Allen, an interesting youth about seventeen years old. . . . Between two and three years before, he had, by a fall from his horse, received a severe injury on his right side, whereby the sixth rib was believed to have been fractured. Considerable ecchymosis immediately appeared . . . no dressing whatever was applied to the wound, . . . The local irritation, and ecchymo-

sis, measurably, though not entirely abated—and during the two years next following, he was frequently able to walk and ride, without serious inconvenience. Several times, however, . . . he was . . . forced to keep his bed for days, and sometimes for weeks. . . . About the end of 1820 . . . a pungent and extremely severe pain was fixed at the vertebral and sternal articulations of the injured rib. . . . A fever of hectic type ensued. . . . A *cessation of pain in the wound* . . . was followed by an *unpleasant feeling of weight and distention*. . . .

On my arrival . . . [to see him] . . . respiration was laborious, with . . . dilatation of the nostrils at each inspiration. . . . No more respiratory motion was perceptible in the right side. . . . The pulse was . . . about one hundred in a minute. The tumefaction of the part was in the direction of the ribs, extending from the sternum to the anterior edge of the latissimus dorsi . . . immediately over the injured part of the sixth rib. . . .

In consultation [with the local physician, Dr. Pugsley] we agreed that one or more ribs were carious, and that an extensive abscess had formed . . . the inward projection of which made such pressure on the right lobe of the lungs, as to impede the function of respiration . . . and prevent the free circulation of blood about the bronchial tubes . . . which only takes place during the inflated state. It was further agreed, that an incision should be made . . . removing such parts of them as might be found carious, and then be governed, in the subsequent part of the operation, by circumstances.

Every necessary preparation being made, at four o'clock the patient was placed supine on a suitable table . . . I commenced the incision at the sternum, and extended it freely through the super-incumbent integuments. . . . A copious discharge followed, not of pus . . . but a kind of grume, and old red coagula. . . .

The sixth rib was found of a caseous consistency. . . . The anterior half of this rib was easily removed by the fingers in small fragments leaving the sternum apparently healthy. . . . A section of the [fifth] rib was removed by cutting forceps, . . . I proceeded to remove the grumous and coagulated matter within the ribs. . . . The depth into the thorax, to which I had now penetrated . . . excited my fears for the safety of the lungs. . . . On making examination with my fingers for the lungs . . . to my utter astonishment and mortification, I was able to *penetrate the right cavity of the thorax, with my first and second fingers, in every direction* to a full depth of three and a half inches, without any resistance, . . . occasionally meeting with more or less disorganized fragments of *bronchial tubes,* all of which tended plainly to prove to me, that *the substance of the right lobe was extensively destroyed*. . . . Fearing to distress the mind of the patient, by making a candid statement of the case to my friend, Dr. Pugsley, I directed the finger of the latter into the wound, and he made a full and satisfactory examination to the same effect. . . . One minute's reflection dictated to me the only course which could possibly be pursued . . . to take away with my fingers, all the parts within my reach which were removable without violence, or the danger of producing hemorrhage. . . . The disorganized parenchyma of the lungs, . . . [was] judged to be between one and two pounds in weight, [after] carefully cleansing it from between and around several of the branches of the bronchial tubes, which appeared to have retained their texture with more tenacity than their surrounding parts. Having done this, I resigned the completion of this part of the operation to nature. . . .

On the thirteenth day his recovery was . . . beyond doubt. . . . From the wound, [there was] a discharge of genuine cream-like pus. . . .

I did not believe it consistent with the powers of nature, for him to survive the immediate effects of the operation. . . . Nor can I believe that the resources of the

system are such as to afford any kind of healthy growth, by which this cavity may be filled and remain well. . . . The structure of the lungs, as well as their function, being entirely unlike that of any other part of the system . . . their vessels, although they *may heal when injured,* have not the power of *reproducing a lost part.* . . .

The only other possible ground of recovery, (if indeed there be one) is the approximation of the sides of the cavity—such as nature would effect in any other part, except the head. This cannot be done, because the arched form of the remaining ribs tends necessarily to keep the cavity from closing by a collapse of its sides. The only curative indication of which I can conceive in the case, therefore, is an unprecedented and desperate resort to surgery, which I should be unwilling to make, and which, most probably, the system would be unable to survive. It is the entire removal of the ribs, from the fifth to the diaphragm, by carefully dissecting them from the muscles and common integuments, leaving these to fall in the cavity, and be applied to its opposite wall for adhesion.

Here was clearly forecast the concept of the thoracoplasty collapse method, which was introduced in the period between 1869 and 1885 by Estlander, Schede, and de Cerenville. Unfortunately, Dr. Antony's patient survived the operative procedure only to succumb on July 11, 1822 of an intercurrent infection or a progression of his old disease.

A quite similar but successful case of Bernard von Langenbeck's (nephew of Conrad) was mentioned by Gluck[39] in 1881.

Five years later (1886) the great Italian internist, Carlos Forlanini, devoted several early paragraphs of his classical monograph on artificial pneumothorax to a consideration of pulmonary ablation as the ideal treatment of tuberculosis.[39] He came to the reluctant conclusion that technical problems of resection were so formidable as to necessitate some more immediately practical therapy and presented his original concept of pulmonary collapse by artificial pneumothorax. However, Tuffier in Paris[48] and Ruggi in Rome[89] essayed the local extirpation of apical tuberculous lesions in several cases with limited success when the lesions were well confined by adhesions. Then the therapeutic pendulum swung heavily toward the collapse procedures, the champions of which, like Brauer and Sauerbruch, were increasingly active at the turn of the century. Resection as a primary method of surgical treatment for phthisis had to await the discovery of streptomycin by Schatz and Waksman[41] and the accumulation of experience with pulmonary ablation in other conditions, chiefly bronchiectasis. In fact, the weight of authority, such as Murphy[80] in America, was definitely against the employment of thoracotomy and resection.

It was in the management of chronic bronchiectasis and its distressing symptomatology that the need for a safe method of resection was particularly pressing and where real progress was first made. Heidenhain[28] described a successful staged partial lower lobectomy in 1901, and sporadic trials

which we shall not attempt to mention in detail took place thereafter in many clinics; more often than not they were abortive or with disastrous results.

Even as early as 1881 the investigations of Gluck[19] and of Block[4] in the laboratory and the later contributions of Biondi,[2] Willard,[46] and Wills[48] were preparing the ground for successful pneumonectomy. However, the lessons of the laboratory were ignored either because the anatomy of the human lung was so at variance with that of small animals, or because pathological changes in the pulmonary hilum seemed to complicate and preclude the ideal technique.[35]

Among American workers in the early years of the present century was Howard Lilienthal[27] who reported in 1922 a personal series of twenty-two lobectomies with a mortality of 45 per cent! This frightening rate was occasioned by a high incidence of bronchial fistula, resultant empyema, wound sepsis, pneumonitis, or occasionally hemorrhage and was not atypical for the times. Rudolf Nissen,[33] once Sauerbruch's first assistant, states that a consecutive series of *ten* lobectomy patients went to their graves before success was first met in that particular German clinic!

Because of high morbidity and mortality rates lobectomy (and pneumonectomy) fell into disrepute among careful and thoughtful surgeons. As has happened more than once in the development of surgery an operation less ideal in concept but more satisfactory in practice came into temporary vogue. This was the multiple-staged cautery pneumonectomy described by Graham in 1925.[20] The initial stage consisted of multiple rib resections over the involved lobes and if necessary an extrapleural wound packing in order to establish pleural adherence and an inflammatory barrier in the chest wall wound. There followed after some days a cautery excision of the more accessible parts of the diseased lobe. The result was a reversal of bronchial drainage through the many external fistulas which inevitably developed. It was a little reminiscent of the procedures described in traumatic cases by Rolando and Fabricius Haldanus. Complications such as delayed hemorrhage, air embolism, empyema, and unsightly chest wall defects sometimes occurred. Withal, the procedure was not as dangerous as the one-stage lobectomy then being performed. Many patients were improved and survived long periods of time. Some subsequently became candidates for completion of lobectomy by modern methods.

In 1927 Wyman Whittemore[45] of Boston voiced his dissatisfaction with the cautery pneumonectomy operation and proposed an alternative procedure. After free entry into the pleural cavity, the involved lower lobe was mobilized. Portions of several overlying ribs were then widely resected to permit the lobe to be partially exteriorized. The operation was terminated by encircling the hilum with a stout catheter and suturing the exteriorized lobe

to the muscles in the wound margin. In about ten days usually the lobe sloughed out. (If not, it could, of course, be further extirpated with a cautery.) Whittemore reported five cases with only one fatality, that from pneumonia. His modest comment on this transitional technique is interesting. ". . . It would seem from a comparison of these two cases [those of Rolando and Tulpius, cited above] with the five reported here, that we have unwittingly come upon a simple technique employed by nature four hundred years ago."

Although Samuel Robertson[36] had recorded five cases of multiple-stage *excision* lobectomy employing mass ligature and suture of the hilar structures with only one death in 1917, the modern era of lobectomy is usually considered to begin with the work of Harold Brunn[6] as reported in 1929. This San Francisco surgeon described six cases, five of lower lobectomy for bronchiectasis and one of right middle and lower lobectomy for cancer with only one fatality. Under *local* anesthesia and with careful dissection to free up the involved lobe, Brunn developed a bronchovascular pedicle which was then doubly occluded with Wertheim clamps and transfixed with heavy catgut sutures. The lobe was amputated distal to the clamps, and the wedged stump oversewn with further catgut sutures. Most important, the pleural cavity and chest wall were entirely closed employing intercostal catheter drainage to minimize residual pneumothorax and effusion. This control of the pleural complications by tube drainage after air-tight closure of the chest was a critical innovation and is still used in the present day.

In Canada, Shenstone and Janes,[49] who were admittedly influenced by the encouraging results of Brunn, provided the second significantly large series from the New World in 1932. Their report comprised 15 cases of single and multiple lobectomy, 14 carried out in a single-stage operation under intercostal procaine block or spinal anesthesia. These writers described the use of a modified tonsil snare to control the lobar pedicle during the amputation of the lobe and subsequent placement of chromic catgut sutures. There were two postoperative deaths from infection and one late death from hemorrhage in the series.

The British Isles were soon represented by Tudor Edwards in 1930-1931[13, 14] and by Roberts and Nelson[37] who summarized ten cases of lower lobe resections for bronchiectasis in 1933. Roberts suggested the possibility of treating chronic abscess by this method but acknowledged that a suitable case had not yet presented itself to his clinic.

In all the preceding the components of the hilum were not individually dissected but were treated as a gross unit by mass ligation or transfixion. This usually resulted in a sloughing necrotic pedicle, bronchial fistula, and acute pyopneumothorax. Indeed, in some early cases the ligatured lobe was

not actually removed at the time of thoracotomy but was allowed to slough away. Such was the technique, crude as it may have been, employed by Rudolph Nissen[21] in performance of the first successful *subtotal* pneumonectomy. His patient, a girl of 12 years, had sustained an injury to the left main bronchus during a crushing accident. Mediastinal abscess and bronchial stricture ensued. Bronchiectasis of the entire left lung was demonstrated by the injection of lipiodol through a fistulous tract in the chest wall. Subtotal pneumonectomy was carried out during July 1931, an elastic ligature supplemented by heavy silk sutures being employed to strangulate the lung, which sloughed out on the 14th day after operation. A bronchial fistula and small empyema healed spontaneously; fortunately, a thoracoplasty was not required. The girl was quite well when presented to the German Surgical Society in 1932 and again in 1934 by Professor Sauerbruch.

Cameron Haight of Ann Arbor[22] was able to report a similar fortunate experience in 1933. His patient, also a girl 13 years of age, was well until she aspirated a foreign body during anesthesia for tooth extraction. Two months later she suffered a spontaneous pyopneumothorax which was drained. Multiple-staged thoracoplasty did not improve her condition. In November 1932 a subtotal left pneumonectomy for bronchiectasis was performed. The pedicles of the two lobes were occluded separately by braided silk ligatures and transfixion sutures, which were left long and extruding through the incision, a technique quite reminiscent of Heister's instructions as laid down in 1724. The upper lobe sloughed away on the 16th day and the lower lobe on the 17th. A protracted but steady recovery period ensued. The patient lived to marry and have at least one child.

Although local extirpations of peripheral lung tumors had been reported previously,[20] it was Evarts Graham[21] who first successfully accomplished a *subtotal* pneumonectomy for cancer of the lung. The patient, a 48-year-old physician and a graduate of Washington University in St. Louis, complained of increasing cough and recurring pneumonitis over a period of seven months. Roentgenograms showed an extensive infiltration of the left upper lobe. A first bronchoscopy was indeterminate, but a second and third yielded a positive biopsy for epidermoid carcinoma. Operation was undertaken on April 5, 1933 with the hope of performing an upper lobectomy for which, of course, there was already ample precedent. It was not possible to encompass the lesion by lobectomy. Therefore the entire lung was mobilized and the hilum temporarily occluded by a catheter. Multiple sutures of #2 chromicized catgut were placed and the lung amputated distal to these. The pulmonary artery stump was further controlled with a separate ligature. Seven 1.5 mc. radon seeds were implanted into the bronchovascular stump. Then a resection of ribs III to XI from the paravertebral region to the mid-

axillary line was performed. Drainage of the pleural space was effected by an intercostal catheter. A bronchopleural fistula followed, leading to empyema which was treated by further drainage. On May 22 additional thoracoplasty was carried out with resection of the first and second ribs. The patient left the hospital on June 18, 1933 and was able to resume his practise in one of our larger Eastern cities. As Doctor Graham himself so modestly stated, all the portents in this famous case were favorable—a small lesion situated at some distance from the primary bronchus, an epidermoid histology, an absence of involved hilar lymph nodes, an otherwise healthy and relatively young man. We might add also, the patient's wise choice of an eminently skillful and courageous surgeon. Nevertheless, the mass ligature-transfixion method of dealing with the pulmonary hilum, while sometimes adequate for dealing with nonmalignant suppurative lesions, was not the final solution in cases of proximally situated tumors nor in elderly poor-risk patients where the complications of bronchopleural fistula and empyema were likely to spell disaster. An accurate anatomical technique was required. This had been shrewdly forecast by Lilienthal[28] in 1925 who wrote in his textbook of thoracic surgery:

> In all of pulmonary surgery it is hard to imagine a condition lending itself better to operative therapy than an early neoplasm of the lung. There is no infection and after extirpation of the tumor-bearing part, healing should be without untoward incident. . . .
>
> In cases of tumor beginning in a bronchus far enough away from the main trunk to permit of amputation proximal to the neoplasm the extirpation of the entire lobe supplied by this bronchus ought to promise at least as good an ultimate prognosis as amputation of the mamma in cancer of the breast. . . . The technique here . . . may be that of the experimental surgeon in which the vessels are caught separately, the bronchus crushed and ligated and disinfected and the entire stump covered in with healthy lung. The thorax would be closed without drainage or with the water-seal drain. . . . Aseptic healing should be possible.

In July 1933 William Rienhoff[29] of Baltimore fulfilled this accurate prediction and accomplished the first *total* pneumonectomy employing an anatomical dissection of the pulmonary artery, the pulmonary veins, and the primary bronchus in their mediastinal courses. The patient was a three-and-a-half-year-old girl afflicted with fibrosarcoma of the left lung. Her convalescence proved a benign one with no clinical evidence of either bronchial fistula or empyema. With this case and a second successful one in November of the same year, Rienhoff clearly demonstrated the superiority of the dissection method and proved that neither thoracoplasty nor postoperative pleural drainage were basic requirements for successful pneumonectomy. The technique employed by Rienhoff was Halstedian in concept and has left little room for other than minor subsequent improvements. Crafoord in Sweden contributed a monograph in 1937.[11]

Once the anatomical feasibility of total pneumonectomy had been established, it was not long before the precise dissection method was applied to the lobar and segmental levels. In 1939 Churchill and Belsey[9] presented a method of segmental resection for the lingula of the left upper lobe. The significance of this work cannot be overestimated because Churchill emphasized that the pulmonary segment rather than the entire lobe is the true surgical and pathological unit of the lung. Bronchiectasis, for example, commonly involves only one or several segments of a lobe while sparing the others in a normal state. This concept became of great practical importance later in the surgical management of tuberculosis.

In 1940 Blades and Kent,[8] working in Graham's clinic, described a technique for the anatomical dissection of the lower lobe hilum, and in 1942[25] presented a detailed study of the surgical problem posed by the other lobes. They prognosticated that precise anatomical dissection methods would be practical for all human lobes with the possible exception of the left upper. This exception, while justifiably cautious, proved not to be necessary.

The systematic anatomical studies of Boyden and his school at the University of Minnesota[5] concerning the bronchial and vascular anatomy of the lungs, both normal and aberrant, are a striking example of the practical results that may be obtained by basic scientists working under the stimulus of an immediate clinical need. Progress in the development of lobectomy and segmental resection techniques has been accelerated not only by Boyden's group but by the clinical contributions of many contemporary surgeons, including Churchill,[10] Overholt,[23] and Chamberlain,[6] to mention only some.

Today no lobe and no segment is exempt from the surgeon's attention by precise, anatomically disposed methods. The excruciatingly slow progress in pulmonary resection since the time of Rolando and Fabricius has been accelerated in tempo so that within the last two decades many of the purely technical problems appear to have been solved.

After having reviewed the history of pulmonary resection, one may perhaps be permitted the luxury of a glimpse into its future. There still remains work to be done on the techniques of conservative bronchial repair, a type of investigation in which Gebauer[18] and others are already active. Somewhat further in the future is the prospect of successful lung grafting or replacement. The requisite methods of bronchial and vascular suture are already available; indeed, it has been demonstrated by Davis and his co-workers[12] that survival as long as eight days after homologous lung replacement can be obtained in the experimental animal. The great obstacle to success, of course, is the host reaction to foreign tissue proteins. When this problem of immunochemistry can be solved, the surgeon will soon thereafter be ready to excise a lung damaged by trauma, infection, neoplasm, or degenerative

disease and replace it with a normal or near-normal one from a homologous donor. This approaches the ideal of surgery, does it not—to take away with the one hand and to restore again with the other? To the ancient aphorism which reads,

> "Ubi pus, ibi incisio; ubi hemorrhagia, ibi ligatura;
> ubi tumor, ibi extirpatio."

the pulmonary surgeon of the future will add still a fourth phrase,

> "Ubi extirpatio, ibi restitutio."

REFERENCES

1. Antony, M.: *Philadelphia med. J.*, 1823, *6*, 108.
2. Biondi, D.: Extirpazione del polmone. *G. Internaz. S. Med.*, 1882, *4*, 759.
3. Blades, B. and Kent, E. D.: Individual ligation technique for lower lobe lobectomy. *J. thorac. Surg.*, 1940, *10*, 84.
4. Block : Experimenteller zur Lungenresektion. *Dtsch. med. Wschr.*, 1881, *7*, 634.
5. Boyden, E. A.: *Segmental anatomy of the lungs. A study of the patterns of the segmental bronchi and related vessels.* New York, McGraw-Hill Book Company, Inc., 1955.
6. Brunn, H.: Surgical principles underlying one-stage lobectomy. *Arch. Surg.*, 1929, *18*, 490.
7. Castiglioni, A.: *A history of medicine.* Translated from the Italian and edited by E. D. Krumbhaar. New York, Alfred A. Knopf, 1941, p. 320.
8. Chamblerlain, J. M. and Ryan, T. C.: Segmental resection in pulmonary disease. *J. thorac. Surg.*, 1950, *19*, 199.
9. Churchill, E. D. and Belsey, R.: Segmental pneumonectomy in bronchiectasis. The lingula segment of the left upper lobe. *Ann. Surg.*, 1939, *109*, 481.
10. Churchill, E. D. and Klopstock, R. R.: Lobectomy for pulmonary tuberculosis. *Ann. Surg.*, 1943, *117*, 641.
11. Crafoord, C.: On the technique of pneumonectomy in man. *Acta chir. scand.*, 1938, *81*, suppl. 54, 1.
12. Davis, H. A., O'Connor, J. P., Coloviras, G. J., Jr., and Strawn, D. L.: Homologous transplantation of the lung. Preliminary report of technical studies. *Arch. Surg.*, 1952, *64*, 745.
13. Edwards, A. T.: Discussion of intrathoracic tumors. *Proc. roy. Soc. Med.*, 1930, *23*, 689.
14. Edwards, A. T.: Malignant disease of the lungs. *Brit. med. J.*, 1931, *1*, 129.
15. Fabricius, Wilhelm von Hilden: *Opera quae extant omnia.* Obs. XXXII. De vulnere quodam pectoris, cum abscissione portionis cuiusdam ipsius pulmonis. Frankfurt, 1646, p. 107.
16. Forde, W.: Case of removal of a portion of lung which protruded through a wound, etc. *Medico-Chirurg. Trans.*, vol. II, second series, London, 1838.
17. Galen: *De anatomicis administrationibus,* Book VIII, Ch. X.
18. Gebauer, P. W.: Further experiences with dermal grafts for healed tuberculous stenosis of the bronchi and trachea. *J. thorac. Surg.*, 1950, *20*, 628.
19. Gluck, T.: Experimenteller Beitrag zur Frage der Lungenextirspation. *Berl. klin. Wchnschr.*, 1881, *18*, 645.
20. Graham, E. A.: Pneumectomy with the cautery. *J. Amer. med. Ass.*, 1923, *81*, 1010.

21. Graham, E. A. and Singer, J. J.: Successful removal of an entire lung for carcinoma of the bronchus. *J. Amer. med. Ass.*, 1933, *101*, 1371.
22. Haight, C.: Total removal of left lung for bronchiectasis. *Surg. Gynec. Obstet.*, 1934, *58*, 768.
23. Heidenhain, L.: Ausgedehnte Lungenresektion wegen zahlreicher eitender Bronchiektasien in einem Unterlappen. *Arch. klin. Chir.*, 1901, *64*, 891.
24. Heister, D. L.: *Chirurgie.* Nürnberg, 1724, p. 95.
25. Kent, E. M. and Blades, B.: The anatomical approach to pulmonary resection. *Ann. Surg.*, 1942, *116*, 782.
26. Langenbeck, C. J. M.: *Bibliothek für die Chirurgie.* Gottingen, 1810. Vol. III, p. 245.
27. Lilienthal, H.: Resection of the lung for suppurative infections, with a report based on 31 operative cases in which resection was done or intended. *Ann. Surg.*, 1922, *75*, 257.
28. Lilienthal, H.: *The surgical treatment of thoracic disease.* Philadelphia, W. B. Saunders Co., 1926, pp. 306-307.
29. Lojacono, S.: Translation of Forlanini's original communication on artificial pneumothorax. *Tubercle*, 1934, *16*, 61.
30. Murphy, J. B.: Surgery of the lung. *J. Amer. med. Ass.*, 1898, *31*, 350.
31. Nissen, R.: Exstirpation eines ganzen Lungenfluegels. *Zbl. Chir.*, 1931, *47*, 3003.
32. Nissen, R.: Personal communication, January 1957.
33. Overholt, R. H., Woods, F. M., and Ramsay, B. H.: Segmental pulmonary resection. Details of technique and results. *J. thorac. Surg.*, 1950, *19*, 207.
34. Paget, S.: *The surgery of the chest.* London, 1896, p. 406.
35. Pean, J. L.: Chirurgie des poumons. *Procès-verbaux Congrès français de Chirurgie*, 1895, *9*, Session 72.
36. Rienhoff, W. F., Jr.: Pneumectomy. A preliminary report of the operative technique in two successful cases. *Bull. Johns Hopk. Hosp.*, 1933, *53*, 390.
37. Roberts, J. E. H. and Nelson, H. P.: Pulmonary lobectomy: Technique and report of ten cases. *Brit. J. Surg.*, 1933, *21*, 277.
38. Robertson, S.: The surgery of bronchiectasis, including a report of five completed resections of the lower lobes of the lungs. *Surg. Gynec. Obstet.*, 1917, *24*, 194.
39. Ruggi, G.: *La tecnica della pneumectomia nell'uomo.* Bologna, 1884.
40. Ruysch, F.: *Observations anatomiques et chirurgicales, au nombre de cent.* Obs. LIII, Guérison d'une playe pénétrante dans la poitrine, avec sortie d'une petite portion du poûmon, p. 75. In: Palfyn, J.: *Anatomie chirurgicale.* Paris, 1734, vol. II.
41. Schatz, A. and Waksman, S. A.: Effect of streptomycin and other antibiotic substances upon Mycobacterium tuberculosis and related organisms. *Proc. Soc. exp. Biol., N. Y.*, 1944, *57*, 244.
42. Shenstone, N. S. and Janes, R. M.: Experiences in pulmonary lobectomy. *Canad. med. Ass. J.*, 1932, *27*, 138.
43. Tuffier, T.: Résection du Sommet du poumon droit pour tuberculose pulmonaire. *Procès-verbaux Congrès français de Chirurgie*, 1895, *9*, Session 87.
44. Tulp, N.: *Observationes medicae.* Editio Quinta. Pulmo praecisus, Liber II, Cap. XVII. Leyden, 1716.
45. Whittemore, W.: The treatment of such cases of chronic suppurative bronchiectasis as are limited to one lobe of the lungs. *Ann. Surg.*, 1927, *86*, 219.
46. Willard, D.: Experiments in pneumonectomy and pneumonotomy. *Trans. Coll. Phys. Philad.*, 1891, *13*, 133.
47. Willis, R.: *The works of W. Harvey, M.D.* Sydenham Society Ed. London, 1846-7, pp. 382-384.
48. Wills, L. W.: An experimental study in pneumonectomy and lung suture. *Sth. Calif. Pract., Los Angeles*, 1892, *7*, 167.

FRANK L. ENGEL*

Departments of Medicine and Physiology, Division of Endocrinology, Duke University, Durham, North Carolina

SOME UNEXPLAINED METABOLIC ACTIONS OF PITUITARY HORMONES WITH A UNIFYING HYPOTHESIS CONCERNING THEIR SIGNIFICANCE†‡

The elucidation of the nature and actions of the adenohypophyseal hormones has been a challenging problem which has been yielding to attack only very slowly. It has been recognized for some time that there are several adenohypophyseal hormones at least one of which, growth hormone or somatotropin, has general systemic metabolic actions.[47] All the others, corticotropin, thyrotropin, follicle-stimulating hormone, luteinizing hormone and prolectin or luteotropin, appear to have their actions primarily if not solely on specific target glands. Despite substantial progress in the morphological localization of the pituitary hormones and their chemical purification, we still know very little either about the exact chemical nature of these hormones as they are formed and exist in the gland, or about their properties as they are secreted into the bloodstream. At one time it was believed that all the pituitary hormones were protein in nature but in recent years it has been established that biological activity of at least one—ACTH—may be associated with a polypeptide molecule which is much smaller than was suspected.[4] However, it must be borne in mind that the mere fact that a polypeptide has biological activity does not necessarily imply that this is the molecular form in which the hormone is stored in the gland or is secreted. Nor can we even be certain that the biological activities reported for the purified hormones are necessarily those of the naturally secreted hormones. It must be appreciated that we are dealing with properties of very complex molecules and that the multiple biological activities of the different pituitary hormones probably depend on specific molecular configurations of peptide chains. The chemist might inadvertently alter these in his search for purity, thereby eliminating some

* Professor of Medicine, Assistant Professor of Physiology, Director of Division of Endocrinology.

† Eastman Lecture, University of Rochester, School of Medicine and Dentistry, Rochester, New York, March 29, 1957.

‡ Supported by grants from the American Cancer Society, the National Institute of Arthritis and Metabolic Diseases (A1324), and by Contract No. DA-49-007-MD134 with the Research and Development Division, Office of the Surgeon General, Department of the Army.

Received for publication June 18, 1957.

biological activities or even endowing the molecule with new properties which nature did not intend it to have. In short, in our studies with pituitary hormones we need to keep an open mind and be prepared to meet surprises. We will find it best not to be too bound by preconceived notions as to what the pituitary hormones "ought to do."

In this lecture I propose to describe to you research which has been in progress in our laboratory for the past six years. It began with a very simple objective, that of elucidating the role of the adrenal cortex in ketone body metabolism, but it has led us into some unexpected areas relating to apparent common biological activities of several pituitary hormones. For some time an explanation for these unexpected metabolic actions of ACTH and other pituitary hormones has seemed to be beyond our grasp. Recently, however, new light has been shed on the problem largely through the opportunity of studying some relatively pure samples of corticotropin and fractions thereof. It is my purpose in this lecture to review some of this work and attempt to interpret it in terms of some speculative hypotheses concerning the action of pituitary hormones. The details of these studies will be published elsewhere.[21, 22]

The adrenal cortex and ketosis. Our studies began with a re-investigation of the role of the adrenal cortex in ketosis and were motivated by the fact that the newer knowledge of the pathways of fat synthesis and catabolism and of ketogenesis made it seem likely that a better understanding of adrenal cortex action might be established thereby.[19] I will not speak in detail of this work, much of which is published,[18, 20, 24, 26, 70] since it is not germane to our main topic, but will simply outline the salient features.

In 1948 it was reported by Bennett *et al.* that ACTH had ketogenic activity in the dog and the rat[6, 7, 8] and, although these findings were not uniformly confirmed,[10] it was a prevailing belief that this ketosis was mediated by the action of adrenal cortical steroids.[71] Kinsell *et al.*[44] were probably the first to question this interpretation when they reported that both cortisone and intravenously infused ACTH inhibited ketonemia in man. Our own studies in the rat likewise showed that both cortisone and hydrocortisone suppressed not only fasting ketosis[24] but also that associated with cold exposure[70] and fluoroacetate poisoning.[12] When ACTH was administered four times a day to the rat during a four-day fast on the other hand, no effect on ketonemia was observed, even though there was obvious stimulation of the adrenal cortices, judging from the blood sugar values. The above observations suggested that the predominant action of ACTH in stimulating steroid secretion did not lead to ketosis but left unexplained the reports of ketosis immediately following ACTH injection.

For this reason, observations on the acute effects of an intraperitoneal

injection of ACTH in the rat were made and it was very quickly established that if blood ketone levels were measured immediately after ACTH injection and before the usual adrenal cortical response, a brisk ketonemia could be demonstrated. In addition to the ketosis, there also was a significant fall in blood sugar, an unexpected response to ACTH.[20] We will return to this observation later.

The antiketogenic response to cortisone (and hydrocortisone) and the ketogenic response to ACTH represented a paradox if we began with the premise that the metabolic actions of ACTH are mediated by the adrenal cortex unless (a) ACTH stimulates the secretion of some ketogenic steroid rather than hydrocortisone, since the latter has been shown to be antiketogenic, (b) the ACTH used was contaminated with some other pituitary factor which was ketogenic, or (c) ACTH has extra-adrenal metabolic actions.

Much time and effort were spent attempting to satisfy ourselves that the adrenal cortex was not the mediator of the ketogenic response to ACTH. The key experiment was the demonstration that ACTH has a ketogenic effect in the adrenalectomized rat.[20] Not only was ketosis demonstrable in the absence of the adrenals but again hypoglycemia was induced. Ketosis was demonstrated in both DOCA and the cortisone-maintained adrenalectomized rats. This experiment indicated clearly that the ACTH was itself responsible for the ketosis and hypoglycemia. This leads us to a consideration of the ketogenic and other metabolic activities of pituitary extracts and hormones.

The ketogenic and adipokinetic factor(s). As has been recently reviewed elsewhere,[11, 19] it has been known for a long time that alkaline extracts of the pituitary gland have the property of causing fatty livers, ketosis, and a lowering of the R.Q. The fat mobilizing activity was later dubbed the adipokinetic activity by Weil and Stetten.[89] With the purification of growth hormone, attention was quickly directed to it to determine whether it was responsible for these metabolic activities. It was soon found that purified growth hormone did indeed have these and other activities which are summarized herewith.

Growth hormone stimulates somatic growth as indicated by an increase in weight and length, and by the widening of the epiphyses of the long bones in the hypophysectomized rat. Chemically, growth is apparent in the production of a positive nitrogen balance[91] and in an increased uptake of isotopically labelled amino acids and of S^{35} and P^{32} by various tissues.[3, 14, 18, 28, 47] In addition, a lowering of plasma amino nitrogen has been described[49, 65, 66, 67] and Russell has shown that the conversion of infused amino acids to urea in the nephrectomized rat is decreased by growth

hormone treatment.[65, 66, 67] Associated with the growth-promoting effects and perhaps even primary to them are influences on general metabolism and on fat and carbohydrate metabolism. Mobilization of fat from the periphery to the liver with hyperlipemia and fatty liver occur and an accelerated catabolism of fat and ketosis can be demonstrated.[1] The R.Q. of the fed animal is lowered, reflecting either increased fat catabolism or inhibition of lipogenesis or both. And finally, some striking immediate changes in carbohydrate metabolism occur which are less well known than the later diabetogenic action. The blood sugar level of the fasted animal is lowered;[52] if glucose is given simultaneously, an improved carbohydrate tolerance is demonstrable.[51] Cardiac and skeletal muscle glycogen levels

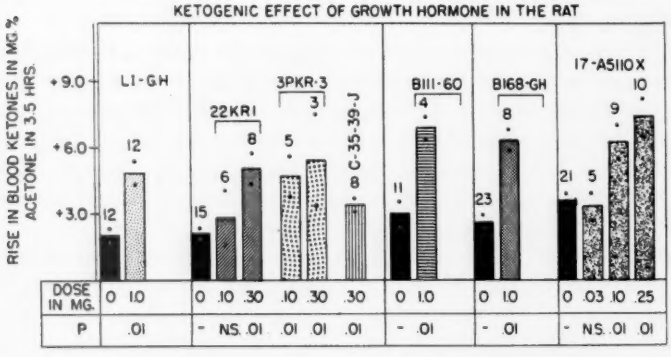


FIG. 1. The ketogenic response to growth hormone following intraperitoneal injection of the hormone in anesthetized rats. The Li, B111-60, and B168-GH were inactive in doses less than 1.0 mg., C-35-39-J in doses less than 0.30 mg.

are increased.[89, 94, 68, 69] With continued growth hormone treatment, diabetes develops secondary to pancreatic islet cell exhaustion.[91]

Now let us return to the problem of ketosis. Having demonstrated that ACTH is ketogenic and hypoglycemogenic in the adrenalectomized rat, we must conclude that either (a) corticotropin is contaminated with growth hormone, or (b) corticotropin has these properties itself and is "the ketogenic factor," thereby contaminating growth hormone, or (c) both hormones have these activities independently. For these reasons, it was necessary to examine a considerable variety and number of samples of pituitary hormone from different sources for their metabolic activities.

Figure 1 illustrates the ketogenic response to a number of samples of bovine growth hormone. The first sample was prepared by Dr. C. H. Li

and kindly supplied to us by him. Samples 22KR1 and 3PKR-3 were old Armour preparations, while the remaining four were prepared by the Wilhelmi technique.[80] B111-60 and B168-GH were supplied to us by Dr. A. E. Wilhelmi and 17-A5110X by Dr. L. Mitchell of the Horner Company. Note that the active doses were of the order of 0.1-1.0 mg. per rat and that the magnitude of the ketogenic responses was modest.

While we were carrying through these studies, Petersen and Lotspeich reported that TSH had greater ketogenic, adipokinetic, and hypoglycemic activity than growth hormone and suggested that these activities in growth hormone could be accounted for by contamination of growth hormone with TSH.[55] For this reason we extended our studies to TSH with the following results (Fig. 2). Note that the ketogenic activities of the two preparations available to us were of the same general order as observed with growth hormone although we did get a response with IRW at 0.040 mg. Triiodothyronine was inactive, suggesting, but not proving, that the ketogenic response was not due to thyroid stimulation.

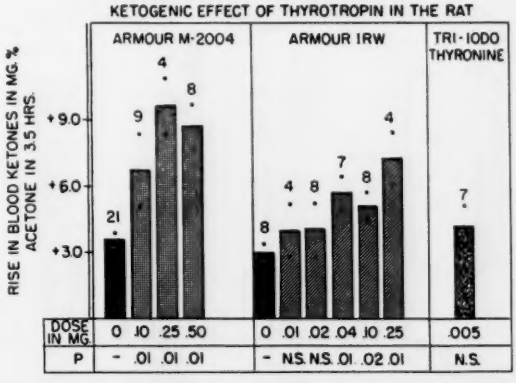


FIG. 2. Ketogenic action of TSH tested as in Figure 1.

In the case of corticotropin, many observations were made, some of which are already published.[18, 20, 25] Figure 3 shows some of the typical responses to the injection of oxycel purified corticotropin obtained from three different laboratories. Note that the minimal active dose is quite low in mg. (0.01-0.03 mg.) and that in general the magnitude of the response is greater than that observed with growth hormone. Note also that mild treatment of the ACTH with alkali did not abolish the ketogenic response, although more severe treatment by boiling in 0.1N NaOH did. We shall return to this observation later.

At the same time as we were conducting these studies, Astwood, Raben, Rosenberg, and Westermeyer were also investigating the metabolic actions of oxycel ACTH.[1, 2, 3, 61, 84] They reported that this material not only was ketogenic, but also had adipokinetic activity, produced hypoglycemia, lowered the R.Q., increased oxygen consumption, and increased cardiac

glycogen, and that all these actions were demonstrable in the absence of the adrenal cortex. Furthermore, studies in our laboratory demonstrated an improved tolerance to intravenously infused glucose[55] and an increased conversion of octanoic acid to ketone bodies in the rat.[25] In short, the studies from the two laboratories described for oxycel ACTH just about all the metabolic activities previously attributed to growth hormone except growth itself.

If oxycel ACTH is indeed contaminated with growth hormone, which seemed unlikely as long as it had greater activity in producing ketosis than did growth hormone, it should be possible to demonstrate the characteristic response of growth hormone, namely, stimulation of growth. The conventional assay for growth hormone depends on the ability of the hormone to promote the widening of the tibial epiphysis in hypophysectomized rats.[58] In the presence of ACTH this assay is unsatisfactory because the glucocorticoids secreted in response to ACTH stimulation inhibit growth of the long bones at the epiphyses. Hence the assays must be done in hypophysectomized-adrenalectomized rats. This is a difficult and tedious procedure. Recently, Russell has developed another method of demonstrating a nitrogen-retaining action of growth hormone.[65] Protein ACTH and the glucocorticoids do not interfere in this technique. This method depends on the fact that growth hormone decreases the conversion of amino acids to urea when they are infused into nephrectomized rats. In 1954, we sent Dr. Russell a sample of Astwood's oxycel ACTH to assay for nitrogen-retaining activity by this technique. Figure 4 shows the results, which were quite unexpected. Oxycel ACTH had clearcut N-retaining activity which

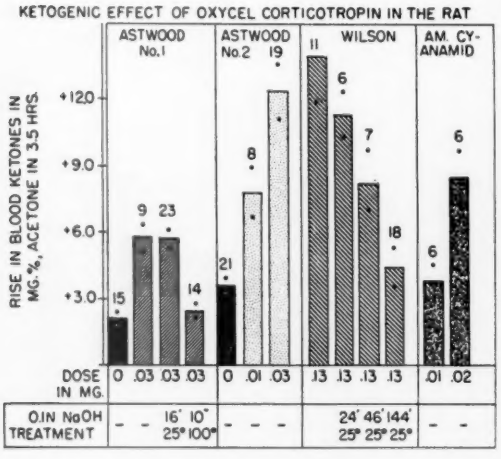


FIG. 3. Ketogenic action of oxycel ACTH tested as in Figure 1. The Wilson ACTH was also tested at a dose of 0.02 mg. and found active. Note resistance to mild alkali treatment, but loss of activity with more vigorous treatment.

was quite comparable to that of an active Wilhelmi growth hormone.* It was difficult to believe that oxycel ACTH had such a great degree of contamination with growth hormone, but at the time the experiment was carried out this conclusion seemed inescapable.[18] Nevertheless, we found this interpretation so difficult to accept that we initiated a series of studies designed to establish whether the ketogenic activities of ACTH, growth hormone, and TSH represented common or independent properties of the

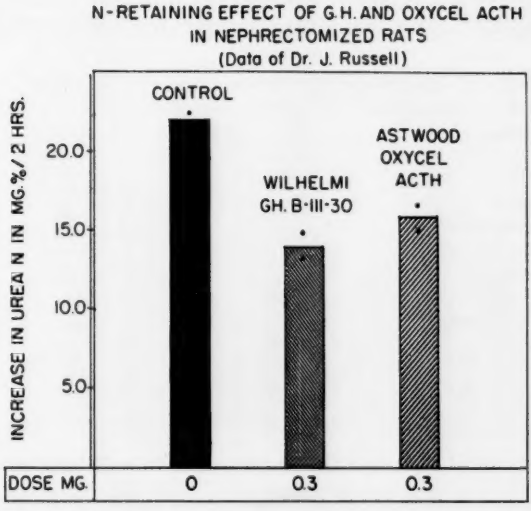


FIG. 4. The N-retaining effect of growth hormone and oxycel ACTH as measured by their ability to decrease the formation of urea N from an infused amino acid mixture in two hours.

three hormones. The urgency of this is apparent when we compare in Table 1 the common activities of the three hormones which have been demonstrated so far.

The activities of growth hormone and ACTH are now pretty well established; those of TSH are on a less firm basis. All three hormones

* This result has now been confirmed in this laboratory. In addition, it has been found that both growth hormone and oxycel ACTH as well as more purified ACTH peptides ($a_1 - a_2$, American Cyanamid) will depress urea formation from infused amino acids in adrenalectomized nephrectomized rats. This metabolic response is thus an extra-adrenal one, but it remains to establish whether it is synonymous with the characteristic protein anabolic and growth-promoting action which is typical of growth hormone.

have been reported to increase the basal oxygen consumption.[1] In the case of TSH, this action is mediated by the thyroid. Growth hormone and TSH both will widen the tibial epiphysis of hypophysectomized rats, but the latter does so by virtue of the stimulating effect of thyroid hormone.[28] ACTH, of course, inhibits growth at the tibial epiphysis because of the anti-anabolic action of the corticoids. All three hormones have been found to lower plasma amino nitrogen levels,[23, 31, 49, 67] although the mechanism and significance of this response remains to be established. Oxycel ACTH and growth hormone will induce N-retention in the nephrectomized rat infused with amino acids. Protein ACTH, however, is inactive.[60] TSH has not

TABLE 1. COMMON METABOLIC ACTIVITIES OF PITUITARY HORMONES

Activities	*STH*	*ACTH*	*TSH*
Increase BMR	+	+	+
Widen epiphyses	+	—	+
Decrease plasma amino N	+	+	+
N-retention-nephrx rat	+	+	?
Fasting hypoglycemia	+	+	+
Increase glucose tolerance	+	+	?
Increase cardiac glycogen	+	+	?
Fat mobilization	+	+	+
Ketogenic	+	+	+
R.Q. depression	+	+	+

been studied. STH and ACTH will increase glucose tolerance and cardiac glycogen.[1, 25, 60] No data are available on TSH. All three hormones will induce hypoglycemia, mobilize fat to the liver, induce ketosis and lower the R.Q.[1, 40] It might be noted in passing that some of these activities are also shared by prolactin.[18]

Attempts to determine whether the ketogenic and adipokinetic activities of these hormones are native to each hormone or represent contamination of one by another have not been wholly successful but have yielded some suggestive results. These will be published in detail elsewhere[21] but are summarized briefly here. First, as noted already, the samples of ACTH were in general active at a much smaller dose than either STH or TSH, making it seem unlikely that the activity of ACTH was due to contamination with the others. At the same time, the reported contamination of the STH and TSH with ACTH was likewise too slight to account for the ketogenic activity of the former. Second, it was found that gentle treatment with alkali (0.1N NaOH for 24 hours at 25°) never decreased the ketogenic and adipokinetic activities of the corticotropins (Fig. 5), but

frequently did abolish these activities in STH and TSH.[9, 15, 21, 48] The mild alkali treatment did not destroy the ascorbic acid-depleting activity of the ACTH but more prolonged or vigorous treatment resulted in loss of both ketogenic and adrenal ascorbic acid-depleting activity (Figs. 3, 5). In addition, it was found by Russell[48] that the mild alkali treatment of oxycel ACTH did not eliminate the N-retaining action of the hormone, but it did destroy this activity in growth hormone. Thyrotropic activity was not tested after alkali treatment. Finally, there was the accidental and unex-

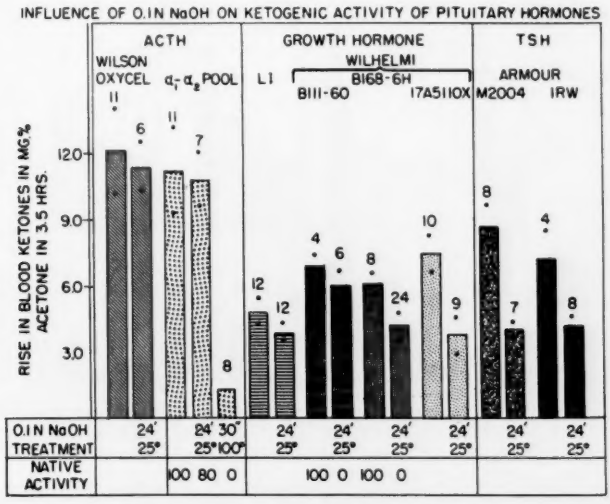


FIG. 5. Mild alkali treatment fails to diminish ketogenic and adrenal ascorbic acid-depleting activities of ACTH but boiling in 0.1N NaOH for 30 minutes does (see Fig. 3). Ketogenic activity of growth hormone B168-GH and 17A5110X was lost, whereas the Li growth hormone and B111-60 resisted alkali treatment. TSH lost ketogenic activity.

plained finding that the ketogenic activity of ACTH was 25-300 fold greater in rats anesthetized with Nembutal, chloral hydrate, or phenobarbital than in unanesthetized rats (Fig. 6), whereas the ketogenic potency of STH and TSH were of the same general order irrespective of whether anesthesia was used or not. Since the Nembutal itself stimulates fasting ketosis, we are inclined to believe that this observation means that ACTH acts at a different locus in metabolism than do the other hormones which stimulate ketosis.

While these various findings are indecisive and subject to a variety of explanations, we feel that they favor the concept that the metabolic

activities in question are intrinsic properties of each of the hormones. Stronger support for this interpretation with relation to ACTH was derived from studies with peptide corticotropins and fractions thereof.

The ketogenic activities of purified corticotropins. Recently the opportunity was presented to us to test purified corticotropins through the generosity of Dr. Paul Bell, who sent us 5 mg. of several samples of ACTH

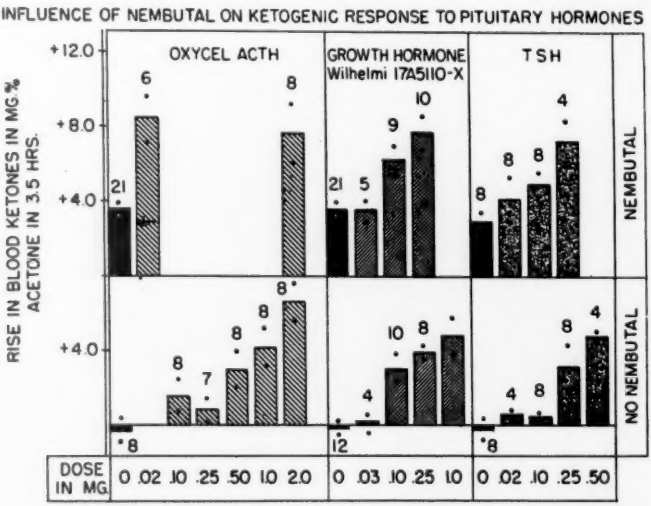


FIG. 6. Comparison of ketogenic responses of ACTH, GH, and TSH in anesthetized and unanesthetized rats. Note that blood ketone levels increase with nembutal anesthesia alone. With ACTH the minimal active dose was 0.02 mg. in the Nembutal group and 0.50 mg. in the unanesthetized. With growth hormone the minimal active dose was 0.100 mg. in both cases, while with TSH it was 0.25 mg. in both cases.

derived from the extensive and elegant characterization studies of ACTH, carried out in the American Cyanamid Company laboratories.[4, 5, 72, 73] Figure 7 shows the activity of four such specimens compared to the original oxycel ACTH. Note that oxycel ACTH, the $a_1 - a_2$ pool and the γ pool all had high activity, while the BP_3 and the δ_{-1} fractions had little or no activity at the highest dose we could test. At the time this study was done we had no idea what these samples represented. Only after Dr. Bell's studies on the chemistry of corticotropin were completed and sent to us could we interpret the data.

Figure 8 presents a flow diagram illustrating the derivation of the samples studied and three activities that have been recorded; ACTH

activity by the adrenal ascorbic acid depletion technique, melanocyte-stimulating hormone activity and ketogenic activity. The first two activities were assayed in Dr. Bell's laboratory. Before describing this fractionation, a brief discussion of the significance of the MSH activity is indicated.

It has been known for a long time that there is a material arising in the intermediate lobe of the pituitary gland which influences pigmentation by dispersal of melanin granules and presumably also by promotion of melanin synthesis.[45, 46] Recently this hormone has been isolated in pure form as α and β MSH and chemically characterized in two laboratories.[29, 30, 32, 32a] A

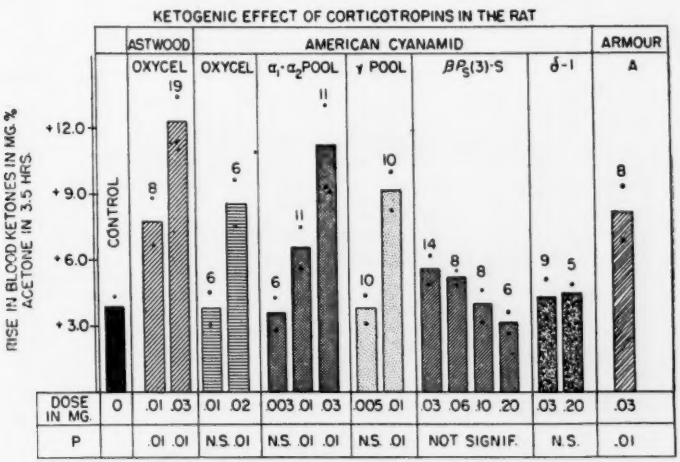


FIG. 7. See text for description.

major puzzle in the last decade has been the regular association of MSH activity with ACTH, an association which led some to suggest that ACTH and MSH were identical.[17, 27, 41, 53, 76–78, 80–82, 90] However, in recent years it has been possible to obtain MSH free of ACTH. As will be seen in the flow diagram and from data to be presented shortly, it now seems apparent that ACTH does have some MSH activity itself, independently of the true melanocyte-stimulating hormone. The significance of this will become apparent shortly.

The starting material in the fractionation was oxycellulose-adsorbed ACTH. This material had ACTH activity of approximately 100 USP units/mg., MSH activity, and was ketogenic at a dose of 0.020 mg. (Fig. 8). By countercurrent distribution, this was separated into a fast moving fraction T6-10 accounting for 80 per cent of the weight, 90 per cent of the

ACTH activity, and 10 per cent of the MSH activity and a slow moving fraction with the remaining activity, i.e., 10 per cent ACTH and 90 per cent MSH, (B1-5). The rapidly moving fraction was further divided into four main fractions, as indicated on the flow diagram. Of these the $\alpha_1 - \alpha_2$ pool was tested by us and found to be ketogenic at a dose of 0.010 mg. Its ACTH and MSH activities were reported to be the same as the starting

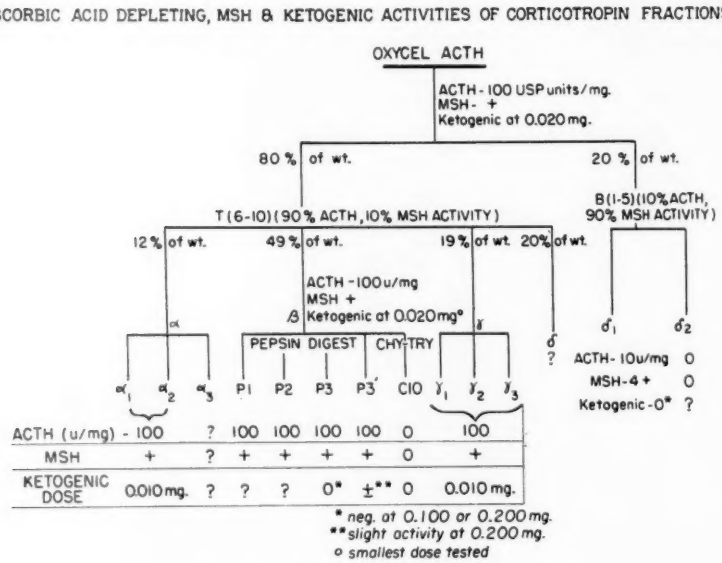

ASCORBIC ACID DEPLETING, MSH & KETOGENIC ACTIVITIES OF CORTICOTROPIN FRACTIONS
OXYCEL ACTH
ACTH - 100 USP units/mg.
MSH - +
Ketogenic at 0.020 mg.
80 % of wt.
20 % of wt.
B(1-5)(10% ACTH, 90% MSH ACTIVITY)
T(6-10)(90% ACTH, 10% MSH ACTIVITY)
12 % of wt.
49 % of wt.
19 % of wt.
20% of wt.
ACTH - 100u/mg
MSH +
Ketogenic at 0.020mg°
PEPSIN DIGEST
CHY-TRY
ACTH - 10u/mg 0
MSH - 4 + 0
Ketogenic - 0* ?
ACTH (u/mg) - 100 ? 100 100 100 100 0 100
MSH + ? + + + + 0 +
KETOGENIC DOSE 0.010 mg. ? ? ? 0* ±** 0 0.010 mg.
* neg. at 0.100 or 0.200 mg.
** slight activity at 0.200 mg.
° smallest dose tested

FIG. 8. See text for description.

material. The largest fraction was designated β-corticotropin. It was highly purified and chemically characterized as a polypeptide containing 39 amino acids. None of this peptide, which again had the same ACTH and MSH activities as the starting material, was available to us. However, Dr. White of the Armour Company kindly supplied us with a sample of corticotropin A which is believed to be identical with β-corticotropin of Bell. The corticotropin A had the same order of ketogenic activity as oxycel ACTH and the $\alpha_1 - \alpha_2$ pool (Fig. 7). Of the four pepsin digestion products of β corticotropin, we were able to test two lots of P3 and one of P4. One sample of P3 showed slight ketogenic activity at 0.200 mg. but was negative at 0.100 mg., while the second was inactive at 0.200 mg. One mg. of P4 was available. It was tested at a dose of 0.200 mg. and found to

have slight activity. Smaller doses could not be tested. Thus P3 and P4 had adrenal ascorbic acid-depleting and MSH activities and much decreased ketogenic activity. The γ pool had high ACTH, MSH, and ketogenic activity. Of the slow moving fraction, δ-1 was tested. This material had much reduced ACTH activity, no ketogenic activity at a dose of 0.200 mg.,

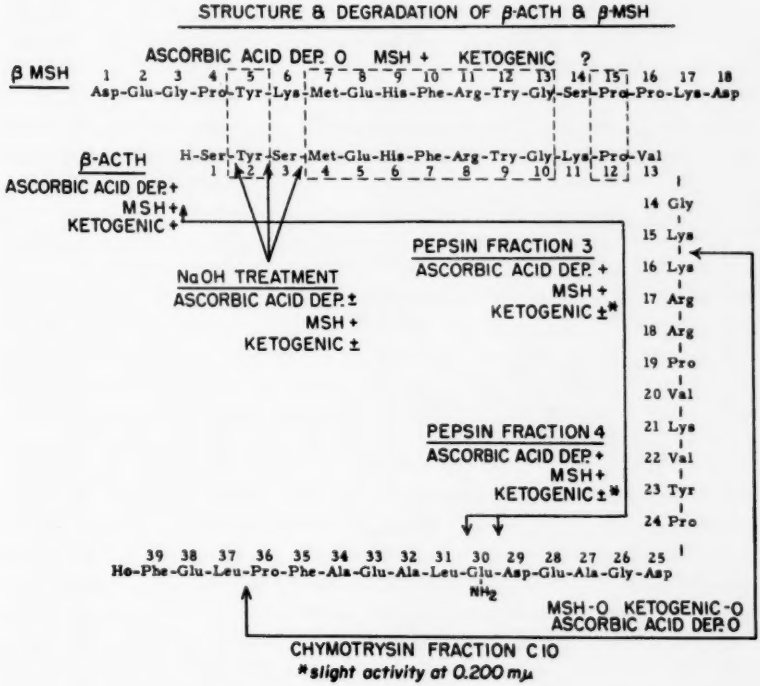


FIG. 9. See text for description.

and much increased MSH activity. It is believed that β-MSH may be an important constituent of this fraction. From these data it is apparent that ketogenic activity still exists in what must be quite pure ACTH polypeptides prepared under conditions in which it is extremely unlikely that there could be significant contamination with other pituitary hormones.

These studies also indicate some dissociation between the structure and function of different corticotropin peptides which is made more apparent in Figure 9 which diagrams the structures of β-MSH as established by Harris and Roos[89] and later by Geschwind *et al.*[50,90] and that of β-corti-

cotropin as characterized by Bell and his collaborators.[4, 5, 72, 73] β-MSH is a polypeptide with 18 amino acids and has in common with β-corticotropin a sequence of seven amino acids as well as two others close by. It seems altogether likely, as suggested by those who determined this structure, that this sharing of amino acids accounts for the MSH activity of the corticotropins, which has been such a puzzle in the past.*

β-corticotropin consists of 39 amino acids with serine as the N-terminal group and phenylalanine as the carboxyl terminal group. This structure is essentially the same, but for minor differences, as the corticotropin peptides characterized by others as corticotropin A and α-corticotropin.[30, 37, 48, 85–88] Moreover, it would appear that the α and γ pools are made up of other peptides with structures similar to but not identical with that of β-corticotropin. In short, there appears to be a family of corticotropin peptides with minor structural differences but similar activities.

In Figure 9 I have attempted to correlate certain information concerning activity and structure. β-MSH has no corticotropic activity but has, of course, strong melanophore-stimulating activity. (A sample of Geschwind and Li's β-MSH has been found to be devoid of ketogenic activity.[23]) Bell's δ-1 fraction, which is presumably rich in MSH, had no ketogenic activity in a dose 10 times that which was active in the α and γ pools of ACTH. Mild alkali treatment did not abolish either ketogenic activity or adrenal ascorbic activity.† Boiling in 0.1N NaOH for 20 minutes, however, resulted in loss of ACTH and ketogenic activities, but, according to the data of Bell, did not abolish MSH activity. Bell's studies indicate that this treatment results in progressive removal of the first three N-terminal amino acids, with progressive inactivation of ACTH. It is noteworthy that this procedure leaves intact the portion of the molecule presumably responsible for the MSH response.[32a]

Pepsin digestion of β-corticotropin does not destroy ACTH and MSH activities, provided at least the first 25 amino acids are left intact. One fraction, P3, consisting of 30 amino acids had no ketogenic activity, while another had slight activity, as did a sample of P4 which consists of 29 amino acids. Since the ketogenic assays were done several years after the original ACTH and MSH assays and insufficient material was left for reassay, these results must be interpreted cautiously. If valid, they represent the only case in which an apparent dissociation between adrenal ascorbic acid depleting and ketogenic action could be demonstrated. Such

* The structure of α-MSH has recently been elucidated by Harris and Learner[32a] and found to have a sequence of 13 amino acids corresponding to those of β-ACTH.

† We are indebted to Dr. George Sayers for the assay of the $\alpha_1 - \alpha_2$ pool for adrenal ascorbic acid depleting activity.

a dissociation has already been noted for MSH and ACTH activities following alkali treatment of ACTH. The finding that the pepsin digest peptide had little or no ketogenic activity led us to wonder whether ketogenic activity might reside in the portion of the molecule which is destroyed by pepsin. We were fortunate to obtain from Dr. Bell a small amount of a chymotrypsin digestion product of β-corticotropin, designated C-10 and comprising the peptide chain from 15-37. This material was known to have no ACTH or MSH activity. On testing, it was found to be devoid of ketogenic activity also.

These studies with essentially pure ACTH peptides leave little doubt that the ketogenic as well as probably the other metabolic activities described for oxycel corticotropin must be native to the corticotropin molecule and as such demand explanation. How can we reconcile the systemic metabolic actions of ACTH with what we know of its action on the adrenal cortex? I think it can be done. What I am now about to present represents a unifying hypothesis concerning certain common and unexplained actions of pituitary hormones.

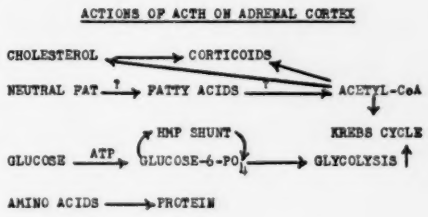


FIG. 10.

A number of attempts have been made to localize ACTH action to one or another locus of action in steroidogenesis,[33, 34, 38, 50, 58, 75] but these interpretations do not seem entirely satisfactory in view of the diverse actions of ACTH which have been recorded. Any concept of ACTH action must reconcile stimulation of growth of the gland (protein anabolism), neutral fat, and cholesterol depletion, ascorbic acid depletion, glycogen loss,[54] increased carbohydrate utilization and oxidation[35, 42, 62] and C19 and C21 steroidogenesis. Figure 10 summarizes some of these responses to ACTH and their possible interrelationships. The cholesterol content of the gland is lowered by ACTH treatment and probably is a source of corticoids. Neutral fat is depleted and in all likelihood is converted to fatty acids and thence to acetoacetyl CoA and acetyl-CoA which are the precursors of steroids. Glucose is utilized by glycolysis and the Krebs cycle,[62] but also there is an active HMP shunt which must be one source of the TPNH necessary for steroidogenesis.[42] Haynes and Berthet[35] have recently indicated that phosphorylase activity is stimulated by ACTH and triggers glycogen breakdown and glucose utilization via the HMP shunt. Finally, as would be anticipated from the growth of the gland, ACTH stimulates

the uptake of C_{14} acetate and amino acids into protein,[26a, 50] and increases the amount of RNA in the gland.[25a] At the moment we can visualize no single mechanism of action of ACTH which would account for all these responses unless it be one involving the reorganization and realignment of the subcellular components of the cell making for a new level of cellular activity.

Irrespective of what might be the mechanism of action of ACTH on the adrenal cortex, it is obvious that many of the metabolic reactions just

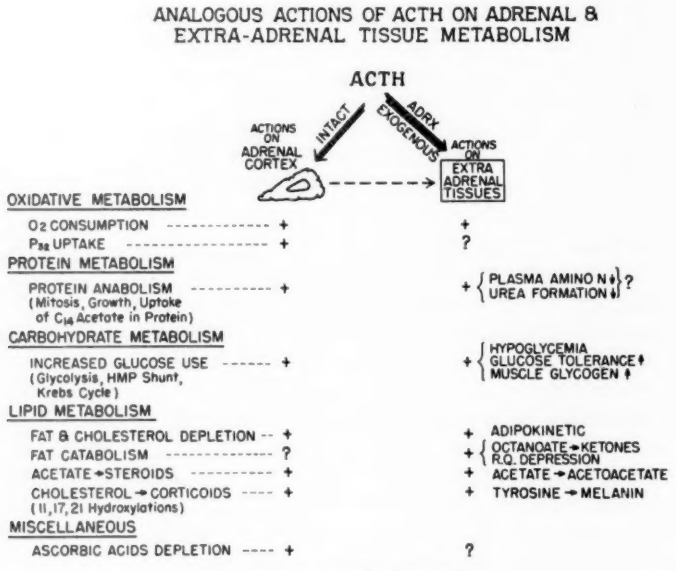

ANALOGOUS ACTIONS OF ACTH ON ADRENAL & EXTRA-ADRENAL TISSUE METABOLISM
ACTH
ACTIONS ON ADRENAL CORTEX
INTACT
ADRX EXOGENOUS
ACTIONS ON EXTRA ADRENAL TISSUES
OXIDATIVE METABOLISM
O2 CONSUMPTION + +
P32 UPTAKE + ?
PROTEIN METABOLISM
PROTEIN ANABOLISM (Mitosis, Growth, Uptake of C14 Acetate in Protein) + + PLASMA AMINO N ↓ UREA FORMATION ↓ ?
CARBOHYDRATE METABOLISM
INCREASED GLUCOSE USE (Glycolysis, HMP Shunt, Krebs Cycle) + + HYPOGLYCEMIA GLUCOSE TOLERANCE ↑ MUSCLE GLYCOGEN ↑
LIPID METABOLISM
FAT & CHOLESTEROL DEPLETION + + ADIPOKINETIC
FAT CATABOLISM ? + OCTANOATE → KETONES R.Q. DEPRESSION
ACETATE → STEROIDS + + ACETATE → ACETOACETATE
CHOLESTEROL → CORTICOIDS (11,17,21 Hydroxylations) + + TYROSINE → MELANIN
MISCELLANEOUS
ASCORBIC ACIDS DEPLETION + ?

FIG. 11. See text for description.

described are analogous to those reported as extra-adrenal activities of ACTH. On the basis of such analogies, a speculative hypothesis is proposed and diagrammed in Figure 11.

It is suggested that under physiological conditions ACTH secreted by the adenohypophysis acts primarily, if not exclusively, on the adrenal cortex, either because (a) this gland has a much greater sensitivity to the hormone or (b) because the gland fixes the hormone specifically, or perhaps (c) because the hormone is secreted as a protein and the adrenal has the most active specific enzyme system necessary to convert ACTH protein to the active peptide. When the hormone is administered in great excess, as in our experiments in which one or more U.S.P. units were used

in the rat (even though the dose was as little as 0.010 mg.), or possibly when it is secreted in excess, as after adrenalectomy, it may exert actions on extra-adrenal tissues. Thus, ACTH stimulates oxygen consumption and P32 uptake[58–60, 74, 79] in the adrenal cortex and reputedly also increases O_2 consumption in the adrenalectomized animal.[1] ACTH stimulates protein anabolism in the adrenal cortex and we have presented evidence that it may, under special circumstances, have an action on extra-adrenal tissues which is identical with one caused by growth hormone. The increase in carbohydrate utilization in the adrenal cortex finds its analogy in the induction of hypoglycemia, increased glucose tolerance, and enhanced cardiac muscle glycogen. The fat "mobilization" and probable fat catabolism in the adrenal cortex are reminiscent of the adipokinetic activity, the increased oxidation of octanoic acid,[25] and the R.Q. depression.* The adrenal cortex converts acetate to steroids. In the liver, acetate is probably converted primarily to acetoacetate under the extra-adrenal influence of ACTH. Acetoacetyl-CoA is considered as a precursor of steroids,[28] but the liver has an active deacylase which favors release of free acetoacetate into the bloodstream and hence ketosis develops.[11, 19] Finally, key steps in the synthesis of corticoids are those of 11, 17, and 21 hydroxylations which have been shown to involve actual transfer of oxygen.[58] It is of more than passing interest that the first step in melaninogenesis, the conversion of tyrosine to DOPA, involves an identical type of oxygen transfer.[45] It is tempting to speculate that the increase in pigmentation in Addison's disease may be based on a perverted action of ACTH resulting in a stimulation of melanin synthesis in the skin instead of corticosteroidogenesis. It is interesting to note in passing that many of the above reactions attributed to the actions of ACTH on extra-adrenal tissues would be neutralized by the action of hydrocortisone and hence would not be anticipated to be operative except in the absence of the adrenals or immediately after a very large dose of ACTH in the intact animal and before the biological actions of the secreted steroids would be manifest.**

---

* Recent, unpublished observations show that both growth hormone and oxycel ACTH will lower the fat content of the testicular fat pad of the fasted mouse and will increase the glycogen content of the dorsal brown fat pad of the fed rat. Adrenalectomized animals will stimulate the release of free fatty acids from adipose tissue *in vitro*.

** In this regard the recent report of Teodoru[75a] is of interest. This investigator has found a difference in the response of brown adipose tissue of hamsters to cortisone and "stress" both with respect to its metabolism and its response to poliomyelitis virus. He suggests that the initial "stress" response is due to ACTH and can be reproduced with exogenous ACTH while the opposite later reaction to stress is influenced by corticoids and can be mimicked by cortisone treatment. If these observations can be confirmed, they would represent the first example of a presumed extra-adrenal tissue and metabolic response to endogenous ACTH.

The above formulation is, of course, highly speculative and is intended primarily to serve as a stimulus for future investigation. Most of the suggestions made are subject to experimental verification and a number are currently under investigation in this laboratory. Moreover, although most of the experimental work has been directed to corticotropin, the concept that this hormone's specific metabolic stimulating activity may be expressed on tissues other than its target gland would seem applicable to other pituitary tropic hormones. In final analysis, they are all "growth and metabolic" hormones for their target glands, and in this respect it should not be surprising that they share some activities in common with the systemic growth hormone, STH, since each gland, in addition to its specific activities, has general metabolic activities comparable to those of other tissues.

TSH has been studied in greatest detail and we have already described certain actions in common with ACTH and STH, but the basis for these actions is less readily discernible. TSH has a well-known extrathyroidal action on connective tissue and the orbit in myxedema and presumably in thyrotropic ophthalmopathy. Since thyroid colloid and myxedema protein contain the same amino sugars,[15] one might speculate that the accumulation of hyaluronic acid in the orbit and elsewhere in myxedema and thyrotropic ophthalmopathy represents a perverted extrathyroidal action of TSH similar to that which we postulated for the action of ACTH in skin pigmentation in Addison's disease.* Prolactin, which is an anabolic hormone for the breast, also has some actions in common with STH.[15] It is intriguing that prolactin is reported to increase the width of the tibial epiphysis of the hypophysectomized male rat but not the female.[28] Does the male animal have less "target" tissue for prolactin to act upon? Diligent search will undoubtedly uncover other examples of common actions of pituitary hormones.

In conclusion, I would like to emphasize again that the elucidation of some of the extra-adrenal actions of ACTH may depend on the fact that the chemists have made available active peptides which might elicit some of these actions more readily than a native protein hormone. In addition, these studies with the biologically active corticotropin peptides indicate that we are on the threshold of a whole new field in endocrinology, the correlation between hormone action and peptide structure. We can now envision the possibility of tailoring peptide molecules to yield specific

* This suggestion is made less attractive by the recent demonstration by Gabrilove and Ludwig[35b] that mucopolysaccharide accumulates in the skin in both primary and secondary (pituitary) myxedema and in each case can be eliminated by thyroid treatment.

metabolic activities. It is not at all unreasonable, for example, to envisage the possibility of deriving from ACTH, or even synthesizing, a peptide which has growth activity but not steroidogenic activity. The future holds much promise in this new field.

REFERENCES

1. Astwood, E. B.: Growth hormone and corticotropin. In: *The hormones, physiology, chemistry and applications.* Pincus, G. and Thimann, K. V., Eds. New York, Academic Press, 1955, pp. 3, 235.
2. Astwood, E. B.: Relation of the metabolic effects of corticotropin concentrates to growth hormone. In: *The hypophyseal growth hormone, nature and actions.* Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds. New York, Blakiston, McGraw-Hill, 1955, pp. 286-292.
3. Astwood, E. B., Raben, M. S., Rosenberg, I. N., and Westermeyer, V. W.: Metabolic effects of a pituitary extract. *Science,* 1953, *118,* 567.
4. Bell, F. H.: Purification and structure of β-corticotropin. *J. Amer. chem. Soc.,* 1954, *76,* 5567.
5. Bell, P. H., Howard, K. S., Shepherd, R. G., Finn, B. M., and Meisenhelder, J. H.: Studies with corticotropin. II. Pepsin degradation of β-corticotropin. *J. Amer. chem. Soc.,* 1956, *78,* 5059.
6. Bennett, L. L., Kreiss, R. E., Li, C. H., and Evans, H.: Production of ketosis by the growth and adrenocorticotropic hormones. *Amer. J. Physiol.,* 1948, *152,* 210.
7. Bennett, L. L., Garcia, J. F., and Li, C. H.: Dose level and degree of ketonemia produced by growth hormone and adrenocorticotropic hormone. *Proc. Soc. exp. Biol., N. Y.,* 1948, *68,* 349.
8. Bennett, L. L., Garcia, J. F., and Li, C. H.: Production of increased ketenemia in a normal dog by adrenocorticotropic hormone. *Proc. Soc. exp. Biol., N. Y.,* 1948, *69,* 52.
9. Bogdonoff, M. D., Scherr, E. H., Lister, L., Owen, J. A., Jr., and Engel, F. L.: A method for the study of the diabetogenic activity of pituitary growth hormone preparations in the rat. *Endocrinology,* 1955, *57,* 272.
10. Bondy, P. K. and Wilhelmi, A. E.: Effect of hormones upon the production of ketone bodies by rat liver slices. *J. biol. Chem.,* 1950, *186,* 245.
11. Campbell, J. and Best, C. H.: Physiologic aspects of ketosis. *Metabolism,* 1956, *5,* 95.
12. Cole, B. T., Engel, F. L., and Fredericks, J.: Sodium fluoroacetate diabetes: Correlation between glycemia, ketonemia and tissue citrate levels. *Endocrinology,* 1955, *56,* 675.
13. Cowie, T. and Folley, S. J.: Physiology of the gonadotropins and the lactogenic hormone. In: *The hormones, physiology, chemistry and applications.* Pincus, G. and Thimann, K. V., Eds. New York, Academic Press, 1955, pp. 3, 309.
14. Daughaday, W. H., Murphy, W. R., and Hartnett, G.: Effect of hypophysectomy and growth hormone on incorporation of S^{35}-sulfate into epiphyseal cartilage. *Fed. Proc.,* 1955, *14,* 34.
15. Deiss, W. P.: Personal communication.
16. Denke, C. W. and Bergenstal, D. M.: The effect of hypophysectomy and growth hormone on S^{35} fixation in cartilage. *Endocrinology,* 1955, *57,* 76.
17. Dixon, H. B. F.: The melanophore-stimulating activity of corticotropin. *Biochim. Biophys. Acta,* 1956, *19,* 392.
18. Engel, F. L.: Factors involved in the ketogenic action of growth hormone. In: *The hypophyseal growth hormone, nature and actions.* Smith, R. W., Jr.,

Gaebler, O. H., and Long, C. N. H., Eds. New York, Blakiston, McGraw-Hill, 1955, pp. 344-362.

19. Engel, F. L.: The influence of the endocrine glands on fatty acid and ketone body metabolism. *Amer. J. clin. Nutr.*, 1957, *5*, 417.
20. Engel, F. L. and Engel, M. G.: The influence of corticotropin on ketonemia and glycemia in normal and adrenalectomized rats. *Endocrinology*, 1954, *55*, 845.
21. Engel, F. L., Engel, M. G., and McPherson, H. T.: Ketogenic and adipokinetic activities of pituitary hormones. *Endocrinology*, In press.
22. Engel, F. L. and Engel, M. G.: The ketogenic activity of corticotropin. A presumed extra-adrenal action. *Endocrinology*, In press.
23. Engel, F. L. and Engel, M. G.: Unpublished observations.
24. Engel, M. G. and Engel, F. L.: Fasting ketosis in the adrenalectomized and cortisone-treated rat. *Endocrinology*, 1954, *55*, 593.
25. Engel, M. G. and Engel, F. L.: Influence of oxycel corticotropin on fatty acid and glucose metabolism in the rat. *Endocrinology*, 1956, *58*, 808.

25a. Fiala, S., Sproul, E. E., and Fiala, A. E.: Regulation by anterior pituitary hormones of nucleic acids in dependent endocrine glands. *Proc. Soc. exp. Biol., N. Y.*, 1957, *94*, 517.

26. Friedberg, F. and Greenberg, D. M.: The effect of growth hormone on the incorporation of S^{35} of methionine into skeletal muscle protein of normal and hypophysectomized animals. *Arch. Biochem.*, 1948, *17*, 193.

26a. Ganis, F. M., Miller, L. L., and Axelrod, L. R.: Effect of ACTH on protein synthesis in isolated perfused beef adrenal gland. *Proc. Soc. exp. Biol., N. Y.*, 1955, *89*, 634.

26b. Gabrilove, J. L. and Ludwig, A. W.: The histogenesis of myxedema. *J. clin. Endocr.*, 1957, *17*, 925.

27. Geschwind, I. I., Reinhart, W. O., and Li, C. H.: Observations on the connexion between intermedin and adrenocorticotropic hormone. *Nature (Lond.)*, 1952, *169*, 1061.
28. Geschwind, I. I. and Li, C. H.: The tibia test for growth hormone. In: *The hypophyseal growth hormone, nature and actions.* Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds. New York, Blakiston, McGraw-Hill, 1955, pp. 28-52.
29. Geschwind, I. I., Li, C. H., and Barnafi, L.: The structure of the β-melanocyte-stimulating hormone. *J. Amer. chem. Soc.*, 1957, *79*, 620.
30. Geschwind, I. I. and Li, C. H.: The isolation and characterization of a melanocyte-stimulating hormone (β-MSH) from hog pituitary glands. *J. Amer. chem. Soc.*, 1957, *79*, 615.
31. Griffin, A. C., Luck, J. M., Kulekoff, V., and Mills, M.: Further observations on the endocrine regulation of blood amino acids. *J. biol. Chem.*, 1954, *209*, 387.
32. Harris, J. I. and Roos, P.: Amino-acid sequence of a melanophore-stimulating peptide. *Nature (Lond.)*, 1956, *178*, 90.

32a. Harris, J. I. and Lerner, A. B.: Amino acid sequence of the α-melanocyte-stimulating hormone. *Nature (Lond.)*, 1957, *179*, 1346.

33. Hayano, M., Saba, N., Dorfman, R. I., and Hechter, O.: Some aspects of the biogenesis of adrenal steroid hormones. *Recent Progr. Hormone Res.*, 1956, *12*, 79.
34. Haynes, R., Savard, K., and Dorfman, R. I.: The action of adrenocorticotropic hormones on beef adrenal slices. J. biol. Chem., 1954, *207*, 925.
35. Haynes, R. C., Jr. and Barthet, L.: Studies on the mechanism of action of the adrenocorticotropic hormone. *J. biol. Chem.*, 1957, *225*, 115.
36. Hays, E. E. and Steelman, S. L.: Chemistry of the anterior pituitary hormones. In: *The hormones, physiology, chemistry and applications.* Pincus, G. and Thimann, K. V., Eds. New York, Academic Press, 1953, pp. 3, 201.

37. Hays, E. E. and White, W. F.: The chemistry of the corticotropins. *Recent Progr. Hormone Res.*, 1954, *10*, 265.
38. Heard, R. D. H., Bligh, E. G., Cann, M. C., Jellinck, P. H., O'Donnell, V. J., Rao, B. C., and Webb, J. L.: Biogenesis of the sterols and steroid hormones. *Recent Progr. Hormone Res.*, 1956, *48*, 423.
39. Illingworth, B. A. and Russell, J. A.: The effects of growth hormone on glycogen in tissues of the rat. *Endocrinology*, 1951, *48*, 423.
40. Iversen, K. and Asboe-Hansen, G.: Studies on the fat-mobilizing factor of the anterior pituitary gland: Suppressive action of thyroxin. *Metabolism*, 1953, *2*, 573.
41. Johnsson, S. and Hogberg, B.: Observations on the connexion between intermedin and adrenocorticotropic hormone. *Nature (Lond.)*, 1952, *169*, 286.
42. Kelly, T. L., Nielson, E. D., Johnson, R. B., and Vestling, C. S., Jr.: Glucose-6-phosphate dehydrogenase of adrenal tissue. *J. biol. Chem.*, 1955, *212*, 545.
43. Kibler, R., Werk, E. E., Jr., Engel, F. L., and Myers, J. D.: A hyperglycemic-glycogenolytic response to pituitary growth hormone in man. *Proc. Soc. exp. Biol., N. Y.*, 1955, *89*, 446.
44. Kinsell, L. W., Margen, S., Michaels, G. D., Reiss, R., Frantz, F., and Carbone, J.: Studies in fat metabolism. III. The effect of ACTH, of cortisone, and of other steroid compounds upon fasting-induced hyperketonemia and ketonuria. *J. clin. Invest.*, 1952, *30*, 1491.
45. Lerner, A. B.: Melanin pigmentation. *Amer. J. Med.*, 1955, *19*, 902.
46. Lerner, A. B. and Takahashi, Y.: Hormonal control of melanin pigmentation. *Recent Progr. Hormone Res.*, 1956, *12*, 303.
47. Li, C. H.: Pituitary growth hormone as a metabolic hormone. *Science*, 1956, *123*, 617.
48. Li, C. H.: Hormones of the anterior pituitary gland. I. Growth and adrenocorticotropic hormone. *Adv. Protein Chem.*, 1956, *11*, 101.
49. Li, C. H., Geschwind, I., and Evans, H. M.: The effect of growth and adrenocorticotropic hormones on the amino acid levels in the plasma. *J. biol. Chem.*, 1949, *177*, 91.
50. Lieberman, S. and Teich, S.: Recent trends in the biochemistry of the steroid hormone. *Pharmacol. Rev.*, 1953, *5*, 285.
51. Massen, A. P.: Growth and metabolism. *Arch. Int. Pharmacodyn.*, 1952, *88*, 434.
52. Milman, A. and Russell, J. A.: Some effects of purified pituitary growth hormone on carbohydrate metabolism in the rat. *Endocrinology*, 1950, *47*, 114.
53. Morris, C. J. O. R., Russell, D. S., Landgrebe, F. W., and Mitchell, G. M.: The melanphore-expanding and corticotrophic activity of human pituitary tissue. *J. Endocr.*, 1956, *14*, 263.
54. Noble, N. L. and Papageorge, E.: Adrenal glycogen of albino rat in various conditions. *Fed. Proc.*, 1953, *12*, 251.
55. Petersen, V. P. and Lotspeich, W. D.: Further aspects of the endocrine regulation of ketogenesis. *Amer. J. Physiol.*, 1955, *182*, 273.
56. Pincus, G.: The biosynthesis of adrenal steroids. *Ann. N. York Acad. Sci.*, 1955, *61*, 283.
57. Raben, M. D. and Westermeyer, V. W.: Recovery of growth hormone in purification of corticotropin. *Proc. Soc. exp. Biol., N. Y.*, 1951, *78*, 550.
58. Reiss, M. and Halkerston, J. M.: Investigations into the phosphorus metabolism of the adrenal cortex. *J. Endocr.*, 1950, *6*, 369.
59. Reiss, M., Brummel, E., Halkerston, I. D. K., Badrick, F. E., and Fenwick, M.: The in vitro action of ACTH on the oxygen consumption of slices of cattle adrenal cortex. *J. Endocr.*, 1953, *9*, 379.
60. Riedel, B. E., Logan, J. E., and Rossiter, R. J.: Phosphorus metabolism of the adrenal gland: Effect of hypophysectomy and administration of ACTH on the

incorporation of radioactive phosphate into phospholipid. *Endocrinology,* 1954, *55,* 219.

61. Rosenberg, I. N.: Adipokinetic activity of oxycel-purified corticotropin. *Proc. Soc. exp. Biol., N. Y.,* 1953, *82,* 701.
62. Rosenfeld, G.: Metabolic studies of intact perfused calf adrenals using tetrazolium. *Arch. Biochem.,* 1956, *62,* 125.
63. Russell, J. A.: Personal communication.
64. Russell, J. A.: Hormonal control of glycogen storage. *Ciba Found. Colloq. Endocr.,* 1953, *6,* 193.
65. Russell, J. A.: Methods of detection and assay of growth hormone. In: *The hypophyseal growth hormone, nature, and actions.* Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds. New York, Blakiston, McGraw-Hill, 1955, pp. 17-27.
66. Russell, J. A.: Effects of growth hormone on the metabolism of amino acids. In: *The hypophyseal growth hormone, nature and actions.* Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds. New York, Blakiston, McGraw-Hill, 1955, pp. 213-224.
67. Russell, J. A.: Hormonal control of amino acid metabolism. *Fed. Proc.,* 1955, *14,* 696.
68. Russell, J. A.: The glycostatic action of purified growth hormone. *Endocrinology,* 1950, *47,* 26.
69. Russell, J. A.: Hormonal control of glycogen in the heart and other tissues in rats. *Endocrinology,* 1956, *58,* 83.
70. Scott, J. L., Jr. and Engel, F. L.: The influence of the adrenal cortex and cold stress on fasting ketosis in the rat. *Endocrinology,* 1953, *53,* 410.
71. Segaloff, A. and Many, A. S.: The role of adrenal steroids and ACTH in gluconeogenesis. A study in phloridzinized animals. *Endocrinology,* 1951, *49,* 390.
72. Shepherd, R. G., Howard, K. S., Bell, P. H., Cacciola, A. R., Child, R. G., Davies, M. C., English, J. P., Finn, B. M., Meisenhelder, J. H., Moyer, A. W., and Van der Sheer, J.: Studies with corticotropin. I. Isolation, purification and properties of β-corticotropin. *J. Amer. chem. Soc.,* 1956, *78,* 5051.
73. Shepherd, R. G., Willson, S. D., Howard, K. S., Bell, F. M., Davis, D. S., Eigner, E. A., Davis, S. B., and Shakespeare, N. E.: Studies with corticotropin. III. Determination of the structure of β-corticotropin and its active degradation products. *J. Amer. chem. Soc.,* 1956, *78,* 5067.
74. Sourkes, T. L. and Heneage, P.: Some studies on the oxidative metabolism of the adrenal gland. *Endocrinology,* 1952, *50,* 73.
75. Stone, D. and Hecter, O.: Studies on ACTH action in perfused bovine adrenals: The site of action of ACTH in corticosteroido-genesis. *Arch. Biochem.,* 1954, *51,* 457.
76. Sulman, F. G.: Chromatophorotropic effect of adrenocorticotropin. A new method for standardization of ACTH. *Vet. Med. Quart. of Israel Vet. Med. Ass.,* 1952, *9,* 31.
77. Sulman, F. G.: Hypersecretion of ACTH chromatophorotropic factor in Addison's disease and its bearing on skin melanosis. *Acta Endocr. (Kbh.),* 1953, *14,* 108.
78. Sulman, F. G. and Evister, A.: The existence of two chromatophore hormones in the pituitary gland. *Acta Endocr. (Kbh.),* 1956, *23,* 120.

78a. Teodoru, C. V.: Influence of cortisone and "stress" upon persistence and multiplication of MEF_1 virus in hamsters. *Proc. Soc. exp. Biol., N. Y.,* 1957, *95,* 674.

79. Tepperman, J.: Effects of purified ACTH added *in vitro* on the oxygen consumption and ascorbic acid content of surviving dog adrenal slices. *Endocrinology,* 1950, *47,* 384.
80. Thing, E.: Melanophore reaction and adrenocorticotropic hormone. *Acta Endocr. (Kbh.),* 1954, *16,* 179.

81. Thing, E., Birch-Andersen, A., and Havn, A.: Melanophore reaction and adrenocorticotrophic hormone. V. On the migration of the melanophore and adrenocorticotrophic activity during paper electrophoresis. *Acta Endocr. (Kbh.)*, 1953, *14*, 113.

82. Thing, E.: Melanophore reaction and adrenocorticotrophic hormone. VI. Comparison of the melanophore and adrenocorticotrophic activity in crude hypophyseal extracts and ACTH preparations. *Acta Endocr. (Kbh.)*, 1954, *16*, 160.

83. Weil, R. and Stetten, D.: The urinary excretion of a fat-mobilizing agent. *J. biol. Chem.*, 1947, *168*, 129.

84. Westermeyer, V. W. and Raben, M. S.: Fall in blood sugar from anterior pituitary extract. *Endocrinology*, 1954, *54*, 173.

85. White, W. F. and Landmann, W. A.: Studies on pituitary adrenocorticotropin. VIII. Synthetic confirmation of three dipeptides from corticotropin A. *J. Amer. chem. Soc.*, 1954, *76*, 4193.

86. White, W. F.: Studies on pituitary adrenocorticotropin. IX. Further sequence near the C-terminus. *J. Amer. chem. Soc.*, 1954, *76*, 4194.

87. White, W. F. and Landmann, W. A.: Studies on pituitary adrenocorticotropin. X. Further sequences near the N-terminus of corticotropin A. *J. Amer. chem. Soc.*, 1955, *77*, 771.

88. White, W. F. and Landmann, W. A.: Studies on adrenocorticotropin. XI. A preliminary comparison of corticotropin-A with β-corticotropin. *J. Amer. chem. Soc.*, 1955, *77*, 1711.

89. Wilhelmi, A. E., Fishman, J. B., and Russell, J. A.: A new preparation of crystalline anterior pituitary growth hormone. *J. biol. Chem.*, 1948, *176*, 735.

90. Winter, C. A., Brink, R. G., and Folkers, K.: Adrenal repair and chromatophore expanding activities of corticotropin-B. *Proc. Soc. exp. Biol., N. Y.*, 1953, *82*, 365.

91. Young, F. G.: Growth hormone and experimental diabetes. *J. clin. Endocr.*, 1951, *11*, 531.

M. C. GOODALL*

Department of Zoology, University of Michigan, Ann Arbor

KINETICS OF MUSCULAR CONTRACTION—I**

The kinetics of muscular contraction is basic for the quantitative description of muscle and understanding its molecular mechanism. However, the subject has been embarrassed by several historical accidents. First, due to the lack until recently of adequate high (mechanical) impedance transducers, attention was focussed on isotonic measurement; this led to the idea of muscle as a shortening generator in series with an elastic impedance—an idea which is quite misleading. Second, the facts surrounding the concept of activation have been confused and oversimplified, partly because of the above, but mainly because the experiments have been done almost exclusively on the frog sartorius which turns out to be a special case, unsuited to elucidating the general picture.

Activation as a precursor to the development of tension originates with Gasser and Hill.[6] By applying a quick stretch at different times relative to the stimulus they showed that

(a) Practically coincident with tension development there is a rise in elastic modulus

(b) After a transient overshoot, the sustained tension, subsequent to such a stretch, occurs earlier than in the unstretched muscle.

The implication from (b) is that early in the onset of contraction a muscle is potentially able to develop some sizeable fraction of its full tension. Thus it appears there are two possible measures of activation:

(1) Potential ability to develop tension

(2) Elastic modulus.

Hill[9-12] has given evidence based on (1) and (2) that activation is established rapidly in comparison with the rate of development of tension. Apparently impressive in relation to (1) is the following[11]: the rate of recovery of tension on quick release to zero from tetanus tension is only 10 per cent higher than that on initial stimulation. This is consistent with his view, which this essentially negative experiment seems to confirm, namely, that activation is a function of time only and is no way influenced

* Research Associate. Present address: Biology Department, Massachusetts Institute of Technology, Cambridge.

** Aided by a grant from Muscular Dystrophy Associations of America.

Received for publication May 27, 1957.

by transient changes of length. However, Hill's result obtained with toad sartorius does not hold in general, for in other muscles Goodall[9] found the increase in rate of recovery may be as much as ten times greater.

Isotonic shortening in the retractor penis muscle of the turtle, Katz[14] found, is faster when delayed from the moment of stimulation, and he concluded: ". . . the full active state is not developed immediately." He also observed the isotonic analogue of the transient: namely, that on a quick increase of load, the muscle takes some hundredths of a second to reach its final rate of shortening.

In their study of the estrogen-dominated rabbit uterus Csapo and Goodall[4] conjectured that activation is rate-limiting. They were unable to show this by the elastic modulus criterion because, as Hill[10] showed, the elasticity in question is nonlinear. However, this merely raises the question whether the increase in elastic modulus on stretch is not an effect on activation itself. In this connection another of Hill's[10] results should be mentioned: on a relatively rapid stretch there is an immediate increase of heat production. Since this cannot be a heat of shortening, it must on Hill's own interpretation be a heat of activation. But this contradicts his assumption that activation is independent of stretch.

Finally, there is the problem of muscles which show maintained oscillations under suitable load (Pringle[18]; Boettiger[1]). That this can also be observed (Goodall[7]; Lorand and Moos[15]) in extracted fibre systems from mammalian muscles, where such oscillations do not normally occur, shows that the mechanism involved must be universally present and any scheme of muscle kinetics must be able to account for it without special assumptions by suitable variation of its parameters.

FORMULATION OF THE PROBLEM

Elasticity. Hill[10] computed the change Δx_e of elastic component x_e of length during isometric tension development for frog and toad sartorii using the characteristic rate equation

$$(3)\ v = -\left(\frac{\delta x_e}{\delta t}\right)_y = k_x a\ (1 - y)\ /\ (a + y)$$

for the contractile component x_e (in Hill's notation the dimensionless variables, $x = x_e + x_c = l/l_0$, $y = P/P_0$, $a = a/P_0$; and the rate constant $k_x = P_0 b/a l_0$. P_0 is tetanus tension, l_0 is rest length, and a is his "shortening" constant). Δx_e is the integral $\int_0^y dy/\,\delta(l, y)$, where the dimensionless elastic modulus

$$\delta\ (x, y) = \left(\frac{\delta y}{\delta x_e}\right)_x = \left(\frac{\delta y}{\delta t}\right)_x / v$$

Hill's results show that $\delta = \delta(y) = \delta(1, y)$ is an increasing function of tension.

This, however, could have been predicted from the observation of Ramsey[17] that for single fibres of frog sartorii,

$$(4)\quad \left(\frac{\delta y}{\delta t}\right)_x = k_y(1-y)$$

from which it follows that

$$(5)\quad \delta = \left(\frac{\delta y}{\delta x_e}\right)_x = \delta_2\ (y+a)/(a+1)$$

where the normalized elastic modulus

$$\delta_2 = \delta\ (1,1) = \left(\frac{a+1}{a}\right)k_y/k_x$$

and (5) has the integral

$$(5)'\quad \delta_2\ \Delta x_e = (a+1)\ \log\ \{(y+a)/a\}$$

for $\Delta x_e = x_e(y) - x_e(O)$.

Hill[11, 18] claimed to have verified the nonlinearity by the direct method of observing y, Δx in a quick release, but this gives the transient elastic modulus $\delta^* = (\delta y/\delta x)_t$ which may not be the same as (5). Actually, his results show that δ^* has the same functional form as (5) but with, in general, different constants; let us say δ_1^*, a^*, which may, however, be functions of the tension y_t from which release was made. They can be calculated from (y, Δx) during a quick release using the relation (5)'.

Activation. We can satisfy both definitions of activation (1) and (2) if we write for the total time derivative of tension

$$(6)\quad dy/dt = k_1(a-y) - v\delta_1 a$$

where a is the activation and the last term expresses the fact that elastic modulus is proportional to it.

The rate equation for activation we shall take as

$$(7)\quad da/dt = k_2(1-a) - v\delta_2 a$$

the molecular basis of this equation is discussed in the Appendix, the last term here indicates that activation can be induced by stretch. The rate (6) is supposed to be switched on at the end of latent period (whose events we are otherwise ignoring here) but (7) may precede it by an interval which we shall calculate.

Eliminating a from (6) and (7) we obtain the operational form

$$(8)\quad (p + k_2 + v\,\delta_2)(k_1 - v\,\delta_1)^{-1}\ (p + k_1)y = k_2 \qquad p = d/dt$$

For the steady state of isotonic shortening, $p = O$ and $v = \text{const.}$ so that (8) becomes

$$(v + k_2/\delta_2)\ (y + \beta\kappa) = (1 + \beta\kappa)k_2/\delta_2$$

which is Hill's equation (3) if we set

$$(9)\ k_2/\delta_2 = a\ k_x,\ \beta\kappa = a,\ \kappa = k_2/k_1$$

The isometric rate equation obtained from (8) by putting $v = O$ is

$$(10)\ (p + k_1)\ (p + k_2)\ (1 - y) = O$$

with general solution

$$(11)\ I - y = Ae^{-k_1 t} + (1 - A)e^{-k_2 t}$$

where the constant A expresses the possibility that (6) may be switched on after (7) in which case there will be an initial activation a_0 and

$$(12)\ A = (a_0 - \kappa)/(1 - \kappa),\ \kappa = \frac{k_2}{k_1}$$

if $k_1 > k_2 = k_y$ the solution (11) reduces to that of (4) asymptotically and for $a_0 = \kappa$ exactly. Thus a $(\log(-y), t)$ plot will tend to a line of slope k_2 intercepting the axis $t = O$ at $\log(1 - a_0)/(1 - \kappa)$. The initial slope will be $a_0 k_1$.

Transient effects. If we eliminate the velocity from (6) and (7), we obtain, using (9),

$$(13)\ d(\beta a - y)/dt = k_1\ (y + a - a(1 + a))$$

putting the right side to zero gives the steady state relation between a and y which we have seen already gives (3). Putting the left side to zero gives the relation for quick changes induced by stretch or release. In particular, we can calculate the initial rate k^* of recovery from a quick release to zero from tension y_t

$$(14)\ k^* = k_1\ (y_t\ (1 - \kappa - \beta^{-1}) + \kappa),\ \text{for } a_0 = \kappa$$

and the rate of recovery when release is from tetanus tension is

$$(14)'\ k^*_y = k_y\ (\beta - 1)/a$$

thus if $\beta > 1 + a$, there will be an increase. The release required to abolish tetanus tension is, from the integral corresponding to (5)', given by $\delta_1 \Delta x_0 = \beta \log\ (1 - \beta^{-1}\overline{1-y})$. From (7) we have in a quick release $\Delta \log a = \delta_2 \Delta x$, so that there exists a release Δx_1 such that activation is reduced to the value k_2/k_1 and then from (11) and (12) $k^* = k_y$. The ratio

$$(15)\ \Delta x_1/\Delta x_0 = \frac{\log \kappa}{\log\ (1 - \beta^{-1})}$$

If we write the isotonic equation in the form

$$(16)\ v = k_x\ (1 - y/a)$$

valid for all times, we notice that the transient effect of the stimulus will not appear at low loads, because effects (1) and (2) of activation just counter-

balance here, nor will it appear at high loads because of the time required to reach the tension involved. The same should apply to the isotonic analogue of quick release, namely, quick removal of load. There should be no increase of rate analogous to (14)′. However, there should be a transient on quick application of load (analogous to quick stretch), the initial rate will be

$$(16)' \quad v^* = k_x\left(1 - \beta \frac{\Delta y}{\Delta y + a\beta/1 + a}\right)$$

that is, zero for $\Delta y = a\beta/(\beta - 1)(1 + a)$.

There is a reservation which must apply to all stretch phenomena; it is well known that (3) does not hold for $y > 1$ and the same may be expected of (7) from its molecular basis. Without adopting a more sophisticated model, the experiments would be consistent with k_2 being reduced to approximately $a\,k_2$ for $a > 1$.

Elastic modulus in quick release. We have on release from tension y_t

$$(17) \quad \delta^* = \delta_1 a = \delta_1(y_t(1 - \kappa) + \kappa - \beta^{-1}(y_t - y)) = \delta_2(y + \beta - 1)/\beta \qquad \text{for } y_t = 1$$

so we have the estimates

$$(18) \quad \delta_1^\dagger = \delta_1,\ a^*\delta_1^\dagger = \delta_2(\beta - 1)(1 + a^*)$$

while considered as a function of y_t at $y = y_t$

$$(19) \quad \delta^*(y_t) = \delta_1((1 - \kappa)y_t + \kappa)$$

Oscillatory phenomena. The second term on the right of (7) has the character of a feedback which may under conditions of inertial load produce maintained oscillations. For small amplitudes in the neighbourhood $a \sim y \sim 1$ we have

$$(20) \quad (p + k_1)\Delta y = k_1 \Delta a - (\omega_0^2/p)\,\Delta y$$

$$(21) \quad (p + k_2)\Delta a = -(\omega_0^2/p)\beta^{-1}\Delta y$$

where $\omega_0/2\pi$ is the resonant frequency with load in the absence of feedback. Solving, we get the secular equation

$$(22) \quad -\omega^2 + i\omega(k_1 + k_2 - (k_1 + \beta^{-1}k_2)\,\omega_0^2/\omega^2) + k_1k_2 + \omega_0^2 = 0$$

so that the condition for maintained oscillations is

$$(23) \quad \omega^2 = k_1k_2 + \omega_0^2 > k_1k_2(a + 1)/(1 - \beta)$$

thus oscillations are possible if $\beta < 1$.

METHODS

Recording was electronic throughout, thermionic transducers (RCA 5734) being used with a blue phosphor C. R. oscillograph (Dumont 304H) which was photographed directly (1:1 magnification) on bromide paper (Kodak 553). Sixty c/s modulation of the beam was used for time register.

TABLE 1

Muscle	*Animal*	*T°C*	k_x	k_y	a *(A)*	κ *(B)*	δ†	δ_s	k_y^*	$\beta-1$	$a^*\delta^*/1+a^*$ *Exp.*	*Calc.*	k_1 *(C)*	*(D)*	*Calc.*	a_0	$k_s\tau_R$
Uterus (estrogen dominated)	Rabbit N. Zealand white	37.5	0.31	2.79[f]	.33[1]	.14	43–108	18	5.4	0.64	...	...	>10	>10	14	.08	.08[1]
R. penis	Turtle (Pseudemys elegans)	0	0.23	0.64	.15	.09	95–135	19	2.2	0.51	9.5	9.7	5	6	6	.03	.16
		10	0.48	1.28	.18	.15	23–110	15	6.7	0.94	12.1	14	....	15	14	.07	.13
R. capitis (Normal)	Turtle (Chrysemys picta)	0	1.05	4.50	.22	.18	70	20	5.4	0.26	4.9	5.2	30.0	....	26	.10	.14
(Ca-depleted)		0	1.25	3.50	.11	.09	46	25	3.5	0.11	3.7	2.8	....	20	35	.05	.11
Sartorius†	Toad	0	0.96	3.80	.21	..	78	20	4.2	0.23	3.1	4.6	....	40	22	.08	.15
	Frog	0	2.00	12.5	.26	..	60‡	24	12.5	0.26	3.2‡	6.2	....	40	60	.11‡	.16

(A) From isotonic data. (B) From length tension in quick release. (C) From kinetics of isometric tension.
(D) From decay of transient on quick stretch. (i, f) Initial, final values.
† From data of Hill.[9–11] ‡ From data of Wilkie.[22]

For isometric recording the muscle was connected by a glass filament and hook via a glass-metal ball joint to a 1:10 reduction lever, mounted on miniature precision ball bearings, to the transducer. The compliance of this system was several hundred times less than the highest recorded in muscle and therefore neglected.

In the isotonic system the load was coupled through 10:1 reduction, and the movement recorded through a small compliance to the transducer. The error introduced by changes in load offered by this compliance was less than 1 per cent.

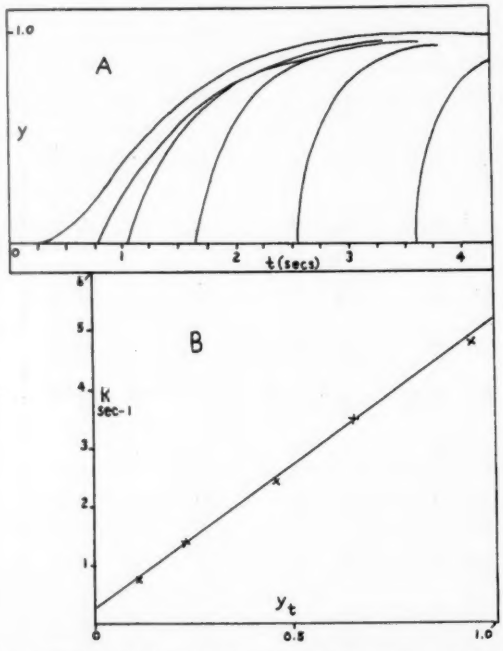


FIG. 1. (A) Isometric tension development; first curve on left without release, subsequent curves from quick releases to zero tension at times, t. (B) Initial rate of recovery k_y^* plotted against tension y_t from which released. Estrogen-dominated rabbit uterus, 37° C.

Quick stretches and releases were finally made by a moving iron transducer giving an average velocity of 200 mm/sec. and operated by an electronic on-off cycle timer synchronized with stimulus. However, for (Δx, y) curves a rack and pinion arrangement with a relay trip mechanism was used, the voltage for the X-plates being derived from a rotary potentiometer coupled to the pinion. The average velocity here was 120 mm/sec. In this arrangement the C.R.O. beam was set at low level until the moment of release (or stretch) when it was intensified by a 30 msec. pulse triggered by the trip relay. The average length of a muscle was 8 cms, so that a 3 per cent release was achieved in a time $\Delta t = 12$-20 msec.

For the rabbit uterus, methods, excepting above, were those used by Csapo and Goodall.[4] For turtle muscles, Krebs solution (Umbreit)[21] modified to Na/K = 50, gassed with 5% $CO_2$95%O_2 which served also as stirring, was used.

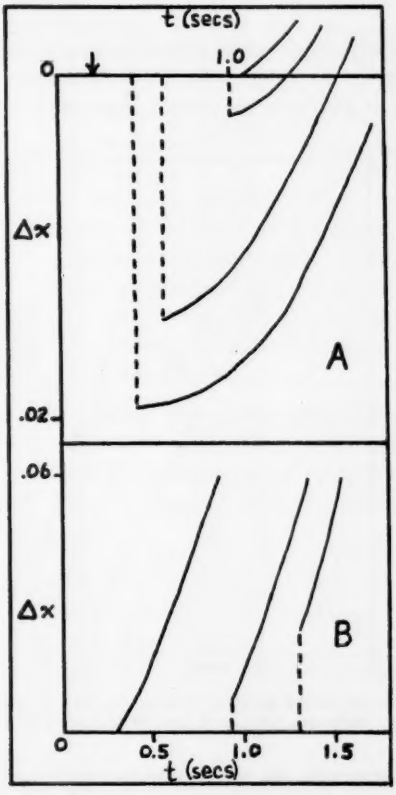


FIG. 2. (A) Shortening after quick application of load y = 0.5 at times indicated. ↓ marks end of latent period. (B) Shortening delayed (by a stop) by amounts indicated. Shows that isotonic rate does not increase with delay as might be expected from results in Figure 1. Dotted lines in either case indicate instantaneous elastic adjustment to load.

Stimulation was effected with homogenous longitudinal field (E) of square pulses (Grass S4B stimulator). A previous study of excitability employing essentially the methods and interpretations of Rushton[19] had been made. The pulse durations τ were held at the point (E τ min.) of minimum energy for muscle (not nerve) excitability. Maximal stimulation, found to occur at 10 x threshold, was routinely used, as was also the optimum repetition frequency. The latter turns out to be about half the reciprocal

latent period and has $Q_{10} \sim 2$. Some experiments were done with transverse stimulation (Brown and Sichel[2]) which established that conduction time effects do not enter into the results.

RESULTS

Survey. The experimental values of measurable quantities referred to in the analysis are given in Table 1 for three essentially different types of muscle, together with Hill's for the sartorii. As a check on our experimental

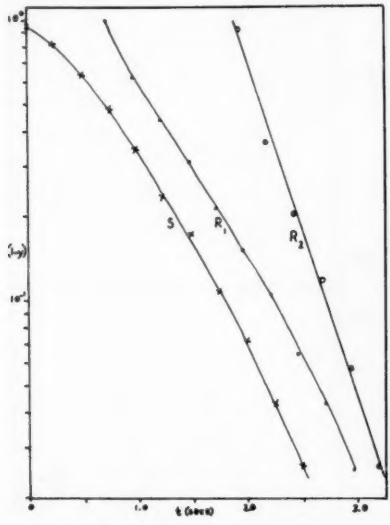


FIG. 3. Logarithmic plot of the curve of Figure 1 (A); S, with stimulation alone; R_1, R_2, release at times indicated. Origin of time shifted to eliminate latent period.

methods we may compare his toad sartorius with our turtle R. capitis (normal) and see that the agreement is satisfactory.

The value of $\delta_2 = k_y/ak_x = 20 \pm 3$ (s. d.) shows a satisfactory constancy. The quantity $(\beta - 1) = ak_y^*/k_y$ which measures the amount of (negative) feedback varies considerably as might be expected and a test of the analysis is to see whether variables derived by independent measurement are in agreement. From (18) we should expect $\delta\dagger$ the normalised elastic modulus to increase with β and it does, but the absolute values are all considerably too high. We shall give reasons for attributing this to an additional parallel visco-elastic component; the values of $a^*\delta^*$, the modulus in the limit of low tension, are in better agreement.

From decay of transients on stimulation, quick release stretch or load we have an independent estimate of k_1 which we can compare with the theoretical value $\beta k_y/a$.

From the initial rate ($= a_0 k_1$) of isometric contraction we obtain the initial activation a_0 to which we may give the following interpretation if τ_A is time delay between beginning of activation and that of tension development then $a_0 = k_2 \tau_A$. For comparison, the corresponding quantity $k_2 \tau_R$ for the latent period τ_R is given in the next column, it is seen that the first is an appreciable fraction of the latter.

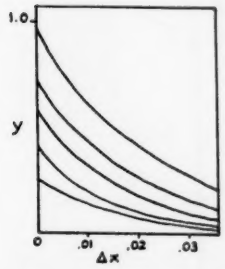


FIG. 4. "Non-linear" elasticity exhibited in quick releases from a range of developed tensions; rabbit uterus, estrogen dominated. Skeletal muscles studies give essentially the same picture, with results given in Table 1.

We have also given the value of κ computed from (19); this is easily measured from the intersection of the tangents at $\Delta x = 0$, for the length-tension curves as given in Figure 5. Considering the uncertainty in the base line for zero tension, these values are in fair agreement with the values of $\beta = a/\kappa$ from k_y^*.

Rabbit uterus (estrogen dominated). This preparation supplies the most striking departure from Hill's "rate of recovery" criterion for rapid activation. Figure 1A shows isometric tension development after quick $\Delta t \sim 30$ msec.) release ($\Delta x = -.05$) to approximately zero tension at times t after the beginning of maximal tetanizing stimulus. The initial rate of Figure 1 recovery against released tension y_t is given in Figure 1B.

Striking though this result is, there are complications with the analysis. The results indicate $(\beta - 1) = a k_y^*/k_y \sim .64$. If we check this with the other data, we have to conclude that k_2 (and therefore a) is increasing with duration of the stimulus.

Figure 2A shows the result of a quick application of load $y = .5$ at times $\Delta t = t - \tau_R$ after the latent period. At the earliest time the rate is reduced just to zero. Using (16) we can estimate what the activation a_t was at this time and so estimate k_2. We have

$$v = k_x(1 - y/(a_t + \beta^{-1}y)) = 0$$

hence

$$a_0 + k_2 t \sim a_t = (1 - \beta^{-1})\Delta y$$

putting in the values $a_0 = .08$, $\Delta t = .2$ sec. we get $k_2 = .57$ sec.$^{-1}$ which is about one-fifth of the final value obtained from isometric data of Figure 3. As a check that k_x ($= k_1 \delta_1$) did not vary, the effect of delayed shortening

at low load was observed. Figure 2B shows the result of delayed shortening for $y = .16$; the increase in rate is less than 25% which is probably accounted for by the fact $y > 0$. We get confirmation of the above conclusion when we look at the $(\log (1 - y), t)$ plot, Figure 3, of the results of Figure 1. The curve S for stimulation alone shows a much slower approach to the final rate than is consistent with any reasonable value of k_1. The curve R_1 shows rapid decay of the increased initial rate which however

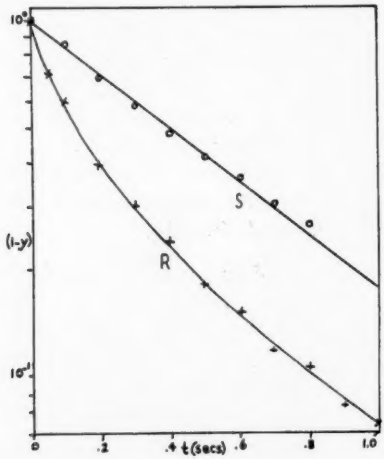


FIG. 5. Same plot as Figure 3, for R. penis (*Pseudemys elegans*), 0° C. Showing that on stimulation alone (S) the asymptotic rate, $k_y = 0.64$ sec.$^{-1}$, is reached instantly, while the increased rate, $k_y^* = 2.2$ sec.$^{-1}$, on release (R) from maximum tension falls to the asymptotic value with a well-defined time constant, $k_1 = 5$ sec.$^{-1}$.

subsequently increases again. The later R_2 shows the initial rate almost maintained.

The method of evaluating a consisted in measuring the initial slope of the load-velocity curve; it will be noticed that the value (from several muscles) is considerably smaller than that obtained by Csapo and Goodall who used the best fit to the whole curve. This is consistent with a and therefore k_2 increasing with time.

Length tension curves in quick release are given in Figure 4. As mentioned earlier, the increase of δ_1^* over δ_2 is larger than expected from (18); however, since all muscles gave a similar result, it was decided to study the details in a muscle which is more easy to handle.

Retractor penis (Pseudemys elegans). All the basic measurements given above were repeated with this muscle. In the [log (1 — y), t] plot for isometric tension Figure 5 curve S gives $k_y = .64$ sec.$^{-1}$ and $a_0 \sim \kappa$. The increased rate, $k_y^* = 2.2$ sec.$^{-1}$, on release from tetanus, cure (R), decays

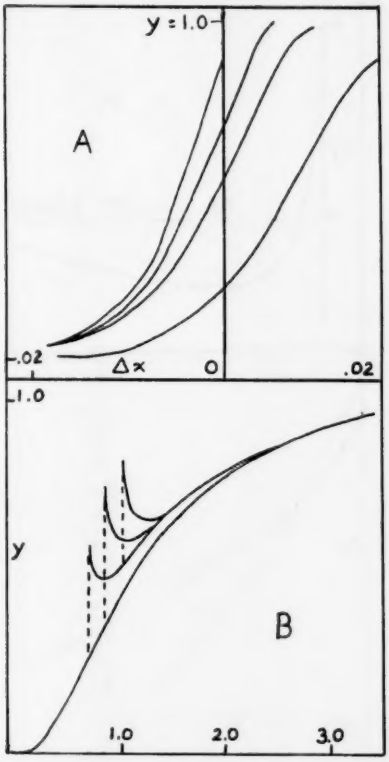


FIG. 6. (A) Showing continuity of elastic modulus between stretch and release, also "saturation" of tension at higher levels. (B) Quick stretches showing decay of transient overshoot to the level associated with the component δ_2 from that associated with δ_1^*.

with rate $k_1 = 5$ sec.$^{-1}$ in good agreement with the value $k_1 = 6$ sec.$^{-1}$ obtained from quick stretch. Similar results were obtained at 10° higher temperature.

With this muscle it is possible to throw some light on why δ_1^* is greater than predicted by (18). First, we show that there is continuity in elastic

modulus between stretch and release, Figure 6A. One notices also that at later times there is an increase in modulus at a given tension. Figure 6B shows the transient following quick stretches, the main part of which

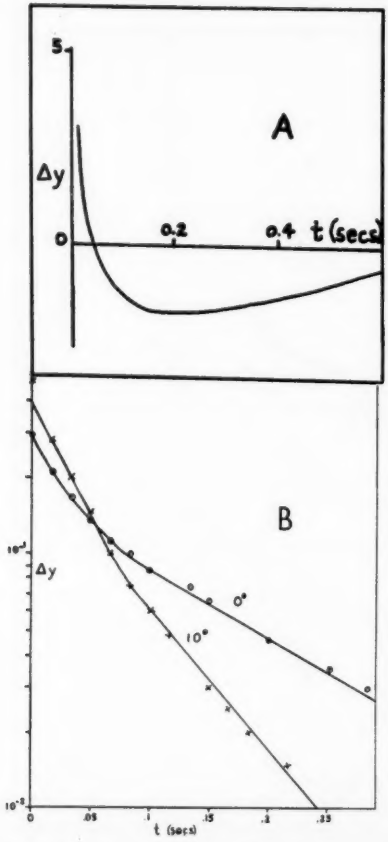


FIG. 7. (A) Transient after quick stretch from near tetanus tension. (B) Logarithmic plot of curves as in (A) at two temperatures showing presence of a rapid temperature independent component.

decays with rate k_1 in agreement with data noted above. However, on closer inspection, Figure 7A, there is a rapid initial part which can be seen more clearly in a (log y, t) plot. This rapid part accounts for all of the excess of δ_1^* over the expected value and furthermore is easily recognized by its

negligible temperature dependence. It increases with the developed tension y_t as can be seen in more detail from Figure 8; A is a plot of log $(y + \alpha^*)$, Δx in quick releases from various y_t, the slopes are δ_1^* and are plotted in B

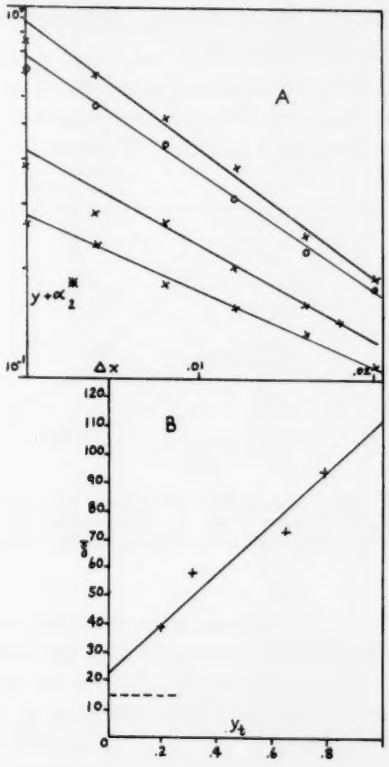


FIG. 8. (A) Logarithmic plot of curves similar to Figure 9(A). ($\Delta x < 0$). (B) Increase in normalized transient elastic modulus δ_1^*, with developed tension. Dotted line approximate value of δ_2; R. penis, T = 10° C.

against y. For small developed tension δ_1^* equals the expected value. One may think of this as a passive parallel (visco-elastic) component and I shall refer to it as such below.

Figure 9 shows the effect of increasing the release (from tetanus tension) above the value Δx_0 required to reduce tension to zero, in this case the stimulus stopped simultaneously with the release so that effects on the sub-

sequent duration of the active state could be observed. The result, namely, that both rate of recovery and duration of active state are diminished, is perhaps the most direct evidence in favour of the postulate embodied in the last term of (7). The value of $\Delta x_1/\Delta x_0$ from (15) is 1.5 for this muscle and the figure shows that the rate of recovery has in fact reached its limiting value k_y at about this level of release.

Retractor capitis collique (chrysemys picta). This muscle is interesting because in solutions containing 2.5 mM Ca^{2+} it resembles, as noted previously, the toad sartorius studied by Hill.[10, 12] The increase in rate of recovery

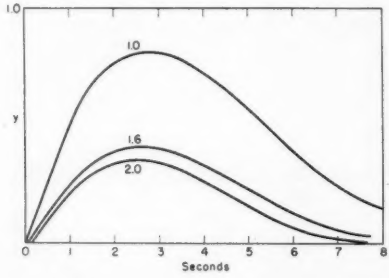


FIG. 9. Showing recovery of tension after releases Δx, from tetanus tension, coinciding with end of stimulus. Numbers refer to release parameter $\Delta x/\Delta x_0$; when the latter $>$ 1 there is delay while the slack is taken up under isotonic conditions. R. penis, 0° C.

on quick release is only 15 per cent, while the values of k_x, k_y are only slightly larger. It is, therefore, significant that the same situation with regard to cumulative effect of stimulus as holds for the rabbit uterus can be induced in this muscle by Ca depletion (24 hours in Ca-free solution, control with Ca shows no change). Figure 10 is the [log $(1-y)$, t] plot. Further, this effect is strongly temperature dependent. Figure 13 gives temperature dependence of the apparent isometric rate constant (measured at the point of inflexion of the (y, t) curve).

Some studies were made with this muscle (normal) of the elastic modulus in the course of a twitch. Figure 12 shows the quotient "δ" $= \Delta y/\Delta x (\Delta x = -.024)$ throughout a cycle. The interesting thing here is that elastic modulus continues to rise after the peak of tension and thereafter falls directly proportional to it. One may hazard the conjecture that the relaxation phase is dominated by the passive parallel component which constitutes in effect a partly deactivated material.

DISCUSSION

It is seen that the sartorius belongs to a group of muscles for which $\beta \sim (1 + a)$ and for which, therefore, most of the effects, apart from the speed, would be unobservable.

The interval $\tau_A = a_0/k_2$ between the switching on of the rate processes (7) and (6) corresponds to the time during which latency relaxation Δy_R is occurring in muscle. One has to conclude that activation per se contributes a negative tension $\Delta y = \gamma a$ where $\Delta y_R = \gamma a_0 \sim 0.1\%$, so that

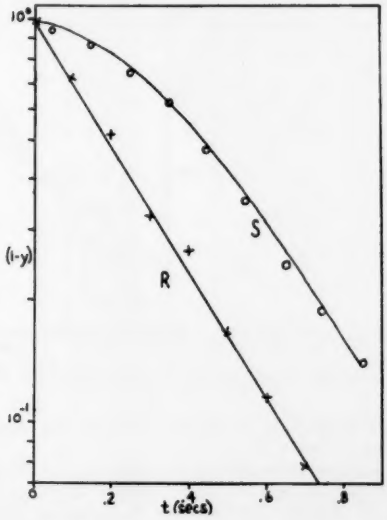


FIG. 10. Logarithmic plot of Ca-depleted R. capitis (*Chrysemys picta*), 0° C.; showing similarity to tension development in Figure 3. S, from stimulation alone; R, released from tetanus.

$\gamma \sim .02$. One can easily verify, adding the appropriate term to (6) and using (7), that this decreases the observable value of δ_1 by an amount $\gamma\delta_2 \sim .04$ which is quite negligible. A more refined treatment of this question is clearly desirable but this would involve among other things something essentially more complicated than equation (7), for which experimental evidence at present is lacking.

The picture which emerges supports the tension generator concept according to which there must be correspondence between chemical states and states of tension rather than of shortening. The conclusion that activa-

tion is rate limiting in all muscles is somewhat revolutionary; however, the evidence is rather direct that this is the case from the fact that muscles which oscillate do so at a frequency some twenty times their isometric rate. This would scarcely be possible if tension generation were rate limiting, and is only so because there is direct coupling between activation and length.

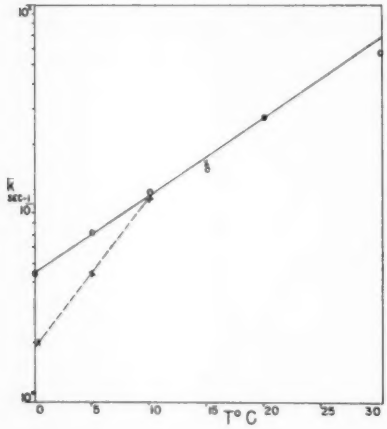


FIG. 11.

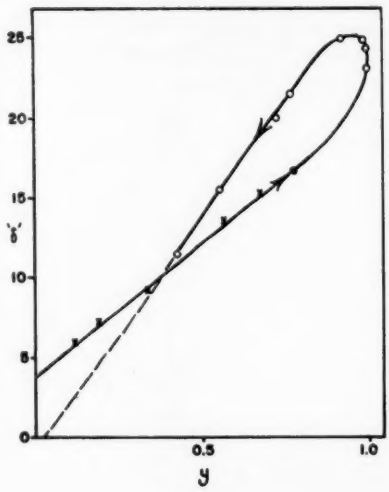


FIG. 12.

FIG. 11. Temperature dependence of isometric rate for Ca-depleted (X) compared to control (O), muscles of Figure 11.

FIG. 12. R. Capitis collique (normal Ca) 0° C', course of elastic modulus "δ" in a twitch plotted as a function of tension (proceeding in direction of arrow). The relatively low values of "δ" obtained here are due to somewhat slower rate of release.

One can give these results a biochemical interpretation (actually the objective of this work) according to the reaction scheme

$$(24)\quad A^{1-a} + M \xrightarrow{ATP\ \ k_2} \underset{a-y}{AM} \xrightarrow{ATP\ \ k_1} \underset{y}{AM}\ ADP^*$$

in which actin (A) and myosin (M) are binding the nucleotides adenosine tri(di)phosphate AT(D)P and the notation * means the presence of mechanical potential energy. The back reactions necessary for relaxation have been omitted since the latter will be dealt with in a following report.

A structural model of contraction has been given by Hanson and Huxley[8] which embodies the essential requirements of a tension generator. That is,

one must have tension proportional to the number of interacting sites between two rigid structures which can transmit it. A. F. Huxley[18] has given a mechanism for this interdigitating model. His is, however, only a first order system which does not account for the facts of activation or elastic modulus and is applied only to obtain an approximation to the steady state isotonic equation (3). His assumptions seem unduly elaborate for the results obtained, and a simpler treatment, interpreting the analysis, is given in the appendix.

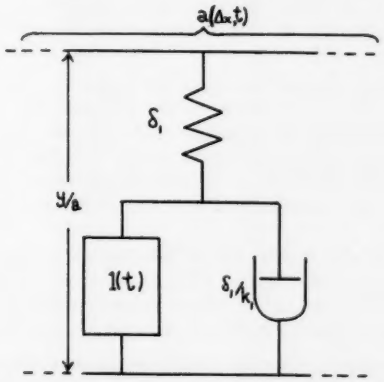


FIG. 13. Equivalent circuit diagram of model site.

SUMMARY

A scheme of kinetics is proposed based on the concept of muscle as a tension generator with the potential ability to develop tension defined as an activation and an elastic modulus proportional thereto. In the rate equation for activation a term is included which describes the effect of length changes on activation. This is a feedback term causing various transient effects and in a particular case oscillations with inertial load.

Parameters occurring in the analysis have been determined experimentally and found to be reasonably consistent. Discrepancies can be accounted for by the presence of a parallel visco elasticity and the cumulative effects of stimulus. The results imply that activation in the sense defined is always rate limiting.

The stimulus transient for isometric tension can be accounted for by a delay between the beginning of activation and that of tension development. This delay is normally about half the latent period, and appears to correspond to the period of latency relaxation.

Ca-depletion of muscle markedly reduces the initial rate constant for activation, which, however, increases with time or the cumulative effect of stimulation.

The feedback term has a natural molecular interpretation in terms of the Huxley-Hanson interdigitating model of contraction. It is shown that the coupling constant, found to be the same for all muscles studied, is equal to the number of interaction sites along the interdigitating distance (approximately half the "A" band in muscle), while the feedback parameter depends on the high energy phosphate potential.

APPENDIX

The mechanical circuit diagram leading to (6) is given in Fig. 13; the number (a) of such elements is given by (7) which includes the statement that each unit tension generator can act only over a given distance after which it is destroyed and is thus associated with an unit of energy of chemical origin. It is the latter assumption which justifies the term tension generator in this connexion, rather than the mechanically equivalent shortening form, for then the concepts of chemical kinetics will apply to the elements.

A model leading to (6) & (7) can be constructed on the following postulates

(1) There is interaction between two rigid linear chains such that their relative motion is a transmitted to the ends (Huxley-Hanson model)

(2) Active bonds (a per unit volume) between chains are formed at sites a distance $\frac{l_0}{\delta_1}$ apart, with rate constant k_2 where l_0 is the interdigitating distance.

(3) Deactivation occurs after reaching adjacent site one and the same side of each site.

(4) Each active bond acquires a tension generator (y per unit volume) of f units with rate constant k_1.

(5) Potential energy of tension generator is supplied by ATP.

(6) Each active bond has a linear elastic modulus of $f\delta_1/l_0$.

Doubtless this model can be given a more sophisticated guise, its importance, however, is that it allows one to attach a meaning to δ_2,β.

Let us first estimate the mol. weight M associated with one site. If f is the tension and l the length and w the wet weight, extracted and intract muscles give maximally

$$fl/w = 3.5 \times 10^8 \text{ gm wt/gm}$$

taking w' the weight of actomyosin to be 15% this gives

$$fl/w' = \frac{.35}{.15} \times 10^7 \text{ ergs/gm} = .55 \text{ Cals/gm}$$

According to Hill the total energy production is

$$dw/dt = k_x(1 + \alpha - y) = k_2/\alpha\delta_2 \cdot (1 + \alpha - y)$$

in the same units as (3), while the rate of formation of sites at zero load is $k_2/(1+\alpha)$ hence

$$Mfl/w' = \alpha\,\delta_2\,\Delta F/(1 + \alpha)^2$$

where $\Delta F \sim 8000$ cals/gm mol is the free energy supplied by ATP. Hence, with $\alpha = .25$, and n mols of ATP acting at each site

$$M = \frac{.8000 \times 3.3}{.55} = 48{,}000n$$

taking the chain length[18] of Myosin as 250 Å/10^5 gm mol we have distance between sites

$$l_o/\delta_2 = 120n \text{ Å}$$

Both electron micrograph[6] and physical chemical data[3] indicate that $n = 3$, so that for $\delta_2 = 20$, $l_o = 7200$ A in agreement with the known half width of the "A" band.

If we assume that the elastic modulus is constant, then $f\beta = \text{const}$, while according to postulate (5)

$$f \propto \Delta F = \Delta F_o + \log[ATP]/[ADP]\,[P]$$

that is the available free energy of ATP which will depend on the presence of a phosphate donor. If we take as "normal" values $\beta = 1.25$, $\Delta F = 8000$ cals/mol, the oscillating region will be reached for $\Delta F > 10{,}000$ which is certainly in the range available from donors such as Creatine phosphate[7] and phosphoenolpyruvate[15] which have been reported to induce oscillations.

REFERENCES

1. Boettiger, E. F. and Furshpan, E.: Triggering the contractile process in insect fibrillar muscle. *J. cell. comp. Physiol.*, 1955, *46*, 370.
2. Brown, D. E. S. and Sichel, F.: Isometric contraction of single muscle fibers. *J. cell. comp. Physiol.*, 1936, *8*, 315-328.
3. Brown, D. E. S. and Goodall, M. C.: Reversal of relaxation mechanism in muscle fibre systems by hydrostatic pressure. *Nature (Lond.)*, 1956, *178*, 1470-1471.
4. Csapo, A. and Goodall, M. C.: Excitability, length tension relation, and kinetics of uterine muscle. *J. Physiol. (Lond.)*, 1954, *126*, 384-395.
5. Gasser, H. S. and Hill, A. V.: Dynamics of muscular contraction. *Proc. roy. Soc. B*, 1924, *96*, 398-437.
6. Goodall, M. C.: Rate of activation in smooth and skeletal muscle. *J. cell. comp. Physiol.*, 1955, *46*, 356-357.
7. Goodall, M. C.: Auto-oscillations in extracted fibre systems. *Nature (Lond.)*, 1956, *177*, 1238.
8. Hanson, J. and Huxley, H. E.: The structural basis of contraction in striated muscle. *Symp. Soc. exp. Biol., N. Y.*, 1955, *9*, 228-264.
9. Hill, A. V.: Heat of shortening and dynamic constants of muscle. *Proc. roy. Soc. B*, 1938, *126*, 136-195.
10. Hill, A. V.: The abrupt transition from rest to activity in muscle. *Proc. roy. Soc. B*, 1949, *136*, 399-420.
11. Hill, A. V.: The series elastic component of muscle. *Proc. roy. Soc. B*, 1950, *137*, 273-280.
12. Hill, A. V.: The mechanics of active muscle. *Proc. roy. Soc. B*, 1953, *141*, 104-117.
13. Huxley, A. F.: Muscle structure and theories of contraction. *Progr. Biophys.*, 1957, *7*, 257-310. §IV.
14. Katz, B.: Relation between force and speed in muscular contraction. *J. Physiol. (Lond.)*, 1939, *96*, 45-64.
15. Lorand, L. and Moos, K.: Auto-oscillations in extracted muscle fibre systems. *Nature (Lond.)*, 1956, *177*, 1239.
16. Pringle, J. W. S.: Mechanism of the myogenic rhythm of certain insect striated muscle. *J. Physiol. (Lond.)*, 1954, *124*, 269-291.
17. Ramsey, R. W.: *Medical physics.* Chicago, Chicago Publishing Company, 1944, pp. 784-798.
18. Rupp, J. C. and Mommaerts, W. F. H. M.: The scattering of light in myosin solutions. II. A determination of the molecular weight. *J. biol. Chem.*, 1957, *224*/I, 277-293.
19. Rushton, W. H. A.: Identification of Lucas' α-excitability. *J. Physiol. (Lond.)*, 1932, *75*, 445-470.
20. Sandow, A.: Latency relaxation and a theory of muscular mechano-chemical coupling. *Ann. N. York Acad. Sci.*, 1947, *47*, 895.
21. Umbreit, W. W.: *Monometric methods.* Minneapolis, Burgess, 1949.
22. Wilkie, D. R.: Mechanical properties of muscle. *Brit. med. Bull.*, 1955, *12*, 177-182.

EVENTS

BOTANY SEMINAR

October 29, 1957

GENETICS OF THE A INCOMPATIBILITY FACTOR OF *SCHIZOPHYLLUM*. By John Raper, Department of Botany, Harvard University, Cambridge, Massachusetts.

Ninety-six distinct compatible A factors have been isolated in Schizophyllum, a basidiomycete. No correlation was found between geographical distribution and the occurrence of these factors. Considerable variation in recombinant classes was found when crosses were made between different strains. This recombination variety was affected by temperature. In addition, many of the recombinants were identical to factors found in nature. This can be explained either by the presence of a single allelic series, or two or more allelic series at nearby loci. In order to elucidate the origin of new incompatibility factors, however, suitable linked markers in the form of biochemical mutants must be found in the region of the known compatibility factors.

H. LAING

MICROBIOLOGY SEMINAR

November 1, 1957

AMINO ACID ACTIVATION AND ITS RELATIONSHIP TO PROTEIN SYNTHESIS. By John A. deMoss, Department of Microbiology, Yale University.

Metabolic energy, in the form of ATP,* is required for protein synthesis from free amino acids. However, experiments with microorganisms revealed that the presence of amino acids is required for the exchange of PP^{32} with ATP in the reaction mixture.

$$PP^{32} + ATP \text{ - - } ATP^{32} + PP$$

The actual concentrations of ATP, PP, and amino acids do not change—which is an indication that the enzymatic system is capable of forming an active intermediate involving the amino acid, and this complex conserves the energy of ATP.

Experiments with extracts of a number of microorganisms revealed that 10 amino acids (in the L form), of a total of 22 tested, were found to promote the exchange of PP^{32} with ATP and suggested that the other 12 amino acids have a different pathway for protein synthesis. Furthermore, fractionation of the extracts suggested that each amino acid is probably activated by a separate enzyme. Hydroxamic acid formation indicated that the amino acids were activated through the carboxyl to form the amino acid complex and amino acid-AMP was proposed as this complex. Leucyl-AMP was also synthesized and found to take part in the reverse reaction of ATP and PP^{32}.

* Abbreviations: ATP—adenosine tri phosphate; AMP—adenylic acid; PP—pyrophosphate.

This direct activation of amino acids might well be the ideal hypothetical first stage of protein synthesis and these activated amino acids could later react with the template system of protein synthesis. Proposals for protein synthesis were discussed.

FAITH N. BRENNEMAN

ANATOMY SEMINAR

November 5, 1957

NEW PERSPECTIVES FOR PROGESTERONE. By Josef Zander, Frauenklinik, University of Cologne, Germany.

The accurate chemical assay of small amounts of steroidal substances has always been a problem. Thiosemicarbazide reacts quantitatively with free carbonyl groups and so can be used to measure steroids with aldehyde or ketone groups. The use of N^{15} labelled thiosemicarbazide to measure chromatographically purified steroid provides a quantitative test which will measure in the order of .001 micrograms of progesterone in recovery experiments. The main obstacle to the widespread introduction of this sensitive test is the problem of purification of biological extracts from interfering carbonyl compounds at its level of sensitivity.

Progesterone plays an important role in the biosynthesis of the corticoids, the androgens, and the estrogens, as well as being an important gestagen itself. *In vitro* studies of progesterone metabolism in the endometrium and uterine muscle produced large yields of the 20α and 20β derivatives of progesterone. The potency of these compounds measured biologically with, for example, the Clauberg test reveals them to be of less potency than progesterone. Since hepatic metabolism of progesterone is largely at the Δ4,3-ketone positions, it is possible that the 20-ketone reduction is either another form of inactivation of progesterone or part of the mechanism of its biological activity or both.

R. N.

ZOOLOGY JOURNAL CLUB

November 18, 1957

METABOLIC ASPECTS OF WAX DIGESTION IN BIRDS. By Herbert Friedmann, Curator of Birds, United States National Museum, Washington, D. C.

Previous work has demonstrated that two microorganisms, a yeast, *Candida* (*-Monilia*) *albicans* and *Micrococcus cerolyticus*, a recently described form, are responsible for lipolysis of ingested wax in the lesser honey-guide, *Indicator minor*, from Africa.

Since honey-guides are hard to obtain alive in numbers, domestic chicks were used for these further experiments carried out in the Department of Bacteriology of the Walter Reed Hospital. Chicks were fed only wax and distilled water inoculated with the two microorganisms. In controls given no microorganisms, there was no change in saponification number (50) of

wax in the droppings. Saponification numbers (which vary inversely with the molecular weight of the fatty acids present) with various inoculations were approximately as follows: *Candida* 100, *Micrococcus* 200, and the two combined 350. The last number shows a synergistic effect, as previously noted in *Indicator*. Warburg respirometer readings were higher for bird intestinal than for *in vitro* cultures, so that an avian "cofactor" exists, enhancing the microorganisms' cerolytic activity. The chick factor is about the same as that for *Indicator*.

It was postulated that the cerolytic microorganisms might have some inhibiting effect on tuberculosis bacilli since *Candida* alone and a combination of *Candida* and *Micrococcus* have been shown to break tubercle wax into fatty acids. Experiments were carried out on both mice and guinea pigs inoculated with doses of tuberculosis normally lethal in six weeks. Mice showed great variability in results, whereas the guinea pigs, which proved more sensitive, gave clear-cut results. Twenty controls (given only lethal doses of tubercle bacilli) were dead within six weeks. Of the twenty experimental animals given bacilli plus the two microorganisms only three died within ten weeks and ten were dead by seventeen weeks. These results compare favorably with those from current antitubercular drugs.

Preliminary experiments with tissue culture inoculations to see if the microorganisms inactivate the bacilli are just beginning to show results. It is also hoped that it can be ascertained whether *Indicator* is tuberculosis immune when more birds become available.

G. E. W. 3RD

MICROBIOLOGY SEMINAR

November 22, 1957

AUTORADIOGRAPHIC STUDIES ON DNA REPLICATION IN BACTERIA. By Frederick Forro, Jr., Assistant Professor of Biophysics, John A. Hartford Research Center, Valhalla, New York.

In the metabolizing cell or bacterium, the majority of evidence indicates that deoxyribonucleic acid (DNA) is not subject to degradation and renewal. However, the question of the integrity of DNA or DNA-containing structures during replication remains open. Studies were performed on *Micrococcus cryophilus* grown on media containing P^{32} in order to incorporate the tracer element into the nucleic acid structure. Radioactivity in individual cells was measured as the number of grains which could be counted after exposure of the bacteria to sensitive radiographic film. Cytochemical extraction procedures were used to determine the proportion of activity attributable to DNA labeling. The distribution of DNA P^{32} among the micromanipulatively isolated progeny of single labeled cells following growth on non-labeled media was non-random.

In more definitive experiments using tritium-labeled thymidine incorporated into the DNA of *E. coli* 15 T^-, the non-random distribution of the labeled DNA was more clearly seen. All-or-nothing partition of the activity between the daughters of a division appeared in the progeny lines at least as early as the third generation. The pattern of distribution in successive

generations indicates the presence in *E. coli* of large DNA-containing structures (as large as 0.5 to 1 x 10^9 in equivalent DNA molecular weight) whose integrity on replication is frequently but not always maintained.

S. H.

ZOOLOGICAL JOURNAL CLUB

December 11, 1957

THE ORGANIZATION CENTER OF THE CHICK BLASTODERM. By Nelson T. Spratt, Jr., Professor of Zoology, University of Minnesota, Minneapolis, Minnesota.

An extensive study of the regressing node area of the chick embryo reinforces the view that the chick possesses an organizer center somewhat analogous to the amphibian dorsal lip.

Techniques of cutting, carbon marking, and vital staining on primitive streak stages grown *in ovo* and *in vitro* have demonstrated the presence of one medial chordal and two lateral somitic organization centers; all three lying in the receding node. The centers function to form axial organs when cells are available immediately caudad to each center; prospective chorda, somite, and lateral plate cells are all pluri-potent with regard to forming any of the axial organs, indicating that what cells normally form may be a function of position only.

Nutritional and cytochemical studies show that cells in the center each possess high basophilia and high dehydrogenase activity. This, in view of the fact that the center has very high glucose requirements and takes up Janus green B extensively, leads to the conclusion that there is a segregation of high numbers of mitochondria in the center. This localization of metabolic machinery permits the high protein synthetic activity of the node and results in what we see as the organization center.

NORMAN K. WESSELS

BIOCHEMICAL DISORDERS IN HUMAN DISEASE. By R. H. S. Thompson and E. J. King, Eds. New York, Academic Press, Inc., and London, J. and A. Churchill, Ltd., 1957. 843 pp. $12.60.

It is becoming increasingly apparent that most diseases are caused by biochemical abnormalities (congenital, nutritional, or acquired through the influence of outside factors such as poisons or microorganisms) and that in those where biochemical abnormalities do not primarily cause the disorder, they play an important role in its course and development. A book purporting to discuss biochemical disorders in human disease should therefore embrace all of medicine and much of surgery. Moreover, since one cannot discuss the abnormal state without a clear understanding of the normal, such a book should also include a comprehensive review of normal biochemistry. This is a large order—perhaps more than can fairly be asked. It is certainly more than has been produced in the present book.

This volume contains 20 essays by 31 authors. The chapters are organized by system (e.g., gastrointestinal, cardiovascular) rather than by disease, although a few diseases (diabetes, porphyrias) have separate chapters. There is no single place where the organized sequence of events leading to energy utilization, substrate dissimulation, or synthetic reactions is discussed. In their preface, the editors say that they feel that the present form of organization will best serve the interests of general medical readers. To me, however, this organization seems wasteful and disjointed. There is considerable passing discussion in the various chapters of the role of high-energy phosphates in energy transport, of the need for coenzymes, of the central role of pyruvate and of acetyl-coA, but there is no single place where the reader can see how these and many other important factors interlock. As a result, the authors labor under an immense handicap. Forsham and Mortimore, in their chapter on Diabetes and Hypoglycemia, recognize this by heading each section with a reference to a standard textbook of biochemistry; but what is the advantage of a book on the biochemistry of disease if it does not contain enough biochemistry to permit the authors to discuss the diseases in biochemical terms? The absence of an introductory section on biochemical integration also causes a good deal of wasted space. The general pattern of biochemical reactions is similar in the liver, kidneys, muscle, gut, brain, etc.; but since there is no single discussion of these similarities, the author of each section is forced to flash a few pertinent fragments of the biochemical pattern before his readers. This causes repetition without integration.

The order of the chapters confounds the confusion, since the chapters on carbohydrate metabolism and nutrition, which might have served as a skeleton on which to hang the details of abnormalities in other systems, are placed in the second half of the book.

Part of my dissatisfaction with this book probably also arises from differing interpretations of the meaning of biochemistry. To me, this implies an investigation of basic mechanisms at a chemical level. Much of the investigation must concern itself with enzymatic reactions, and with the anatomical arrangements within the cell which control or modify these reactions. The influence of genetic abnormalities and substrate storage and transport also would play a large part here. Apparently, not all of the authors in this

symposium agree with my concept. Many of the essays are concerned chiefly with clinical chemistry, as reflected in how to do urine tests or interpret blood studies. Considerable portions of certain chapters are devoted to purely practical applications of clinical tests (e.g. of liver function) without any consideration of their biochemical significance.

In spite of these editorial criticisms, this book has many chapters of great merit. I was especially impressed by D. A. K. Black's discussion of the gastrointestinal tract, MacFarlane's essay on blood clotting, Milne's discussion of renal function and disease, Jailer and Longson on the adrenal, and Wolff and Goldberg on iodine metabolism, to mention a few. Some of the other chapters were less meritorious; indeed, MacLagan's discussion of the liver seemed totally out of place in such a book as this. (I find it difficult to understand how one could discuss biochemical disorders in liver disease without even mentioning that the liver is the chief place in the body where urea is formed, for example). On the whole, although the book does not entirely fulfill its purpose, as I conceive it, the excellence of much of the discussion justifies its use as a reference book, particularly for biochemists and medical students who will find the absence of formally presented biochemical concepts less of a problem than the medical practitioner.

PHILIP K. BONDY

BODY WATER IN MAN. THE ACQUISITION AND MAINTENANCE OF THE BODY FLUIDS. By Maurice B. Strauss. Boston, Toronto, Little, Brown, and Co., 1957. 286 pp. $7.00.

> "The one commonplace of all cell activity is *adaptation*, and we must show that any assumed activity of the cells, besides involving expenditure of energy by the cells, is also orderly and adapted. To call in vital activity as a sort of irresponsible deity to explain irregularities in our experimental results is an unscientific and I might say cowardly device."
>
> —E. H. Starling, *The fluids of the body.*

This is the only dedication appearing in the classic monograph by John P. Peters, *Body Water,* published in 1935. During the intervening two decades, a flood of literature has expanded our knowledge of bodily adaptations dealing with salt and water. In this volume, dedicated to "John Punnett Peters, M.D., 1887-1955, Physician, Scientist, Citizen," Dr. Strauss has reviewed the present status of *Body Water in Man* from the vantage point of one who has been physician as well as physiologist, and who does not disdain the philosophical.

The words "In Man" are important in this exhaustive (but not exhausting) survey of factors influencing the intake and excretion of water and salt. As a clinician, Strauss is keenly aware of the difference between dog and man; as an experimental subject, he can leaven necessarily detailed descriptions of complex experimental procedures with a sharp appreciation of the antidiuretic side-effects of pain and anxiety. On the other hand, the chapter on "Perspectives from cosmogony and comparative physiology" contains a fascinating recapitulation of the regulation of volume and osmotic pressure by invertebrate animals exposed to extremes of fresh and salt water.

Dr. Strauss is aware of the importance to the body of the mechanisms influencing the disposal of water, although his teleology is sometimes tongue-in-cheek. In discussing diurnal variations in urine flow, "the teleologic usefulness of a diminished output of urine during the usual time of sleep requires no comment."

Approximately twice as much space is devoted to thirst, water, and the antidiuretic hormone as to appetite, salt, and aldosterone. This is perhaps appropriate, since much of the work by Dr. Strauss and his colleagues has been directed to clarifying the former field. Certainly there is no more detailed and inclusive summary of current knowledge in this area than appears in this volume. The bibliography has over five hundred references, ranging from the Old Testament (Leviticus II: 13, on the use of salt) to unpublished data obtained in 1956. It is not a criticism of the book, but a rueful comment on the speed of scientific advance, that the recent "countercurrent" theory of Wirz is not mentioned in the chapter devoted to "The Renal Excretion of Water."

Body Water in Man will be welcomed by all students, investigators, and physicians who are interested in the physiology of body fluids.

FRANKLIN H. EPSTEIN

HISTOLOGY. By Arthur W. Ham. Philadelphia and Montreal, J. B. Lippincott Co., 1957. 3d ed. xv, 894 pp. 582 figs. $11.00.

The third edition of this popular book presents a rather complete account of histology in which basic principles are well correlated with newer research findings. The approach is from a physiological viewpoint, which stimulates the reader to think in terms of tissue and organ dynamics, rather than in terms of histological structure alone. The author effectively correlates microscopic appearance with normal and abnormal function, thus introducing the beginning student effectively and enjoyably to later studies of tissue and organ pathology.

This edition has been extensively revised, with the incorporation of a great deal of new data. Many sections, such as that on the liver, have been completely rewritten. The explanation of liver structure and function is excellent and makes this topic clear and lucid to the reader. The coverage of the sense organs, especially the ear, is accurate, concise, and well writtten, as in the previous edition. The endocrine organs are considered in rather great detail; the material presented overlaps, to a large extent, similar chapters in textbooks of physiology. The other organ systems are described in equally readable style. The vertical approach in explaining a given type of tissue is refreshing.

The illustrations are well chosen and well integrated with the text; their direct labelling is a decided convenience. Electron micrographs are used to great advantage. The introductory chapters will be of value to medical students who lack a background in basic cell structure and microtechnique. The bibliographies are complete and enhance the usefulness of the book for reference work. Since this textbook contains a great deal of advanced material, the student in a beginning course might well reserve certain advanced aspects of each topic for reading at a later time. This textbook is recom-

mended as a modern, refreshing account of histology, appealing especially to the beginning medical student.

MALIN R. DOLLINGER

RHEUMATOID ARTHRITIS. By C. L. Short, W. Bauer, and W. E. Reynolds. Cambridge, Mass., Harvard University Press, 1957. 480 pp. $7.00.

This volume is rewarding reading for the clinician as well as the investigator. It represents an exhaustive numerical study of rheumatoid arthritis, gained through the observation of 293 consecutive patients admitted to the medical wards of the Massachusetts General Hospital between 1930 and 1936 with the diagnosis of rheumatoid arthritis. An equal number of controls was selected from patients and hospital employees. All arthritis patients received only simple medical and orthopedic treatment.

Almost a quarter of the book is devoted to the development of the concept of rheumatoid arthritis as a disease entity, and the diagnostic criteria used in the selection of the patients. This is perhaps the most valuable part of the book in that definition of the disease remains one of the most interesting and challenging aspects of clinical investigation today. The reasons for including some clinical forms as variants of rheumatoid arthritis and for excluding others as different diseases are of great interest, and further clarify the authors' concept of rheumatoid arthritis.

Forty chapters cover factors of possible etiological significance, aspects of the disease associated with the onset, its course, factors believed by the patients to have influenced it, physical findings, extra-articular symptoms, and laboratory studies. Paragraphs, tables, and figures have been numbered to permit ready recognition of their chapter location.

The cross-sectional delineation of the disease, and the long-term follow-up study of the course of rheumatoid arthritis give the reader statistical impressions gained from numerical comparisons made between the arthritis patients and the control subjects, and between sub-groups of the arthritis patients, for many variables of clinical interest. Throughout, the authors have kept an eye open for the possible, special significance of each facet of the disease, to determine if it can be shown to be of diagnostic or prognostic help. The book is an excellent reference text for the student of rheumatoid arthritis to pick up in reflective moods. At the close of many chapters, provocative suggestions for future studies are presented by the authors.

The fact that tabulations were made of the patients' disease status in 1937, 1947, and 1954 relative to their condition on hospital admission between 1930 and 1936, offers future investigators a reference of comparison for series of patients receiving special forms of therapy.

The authors are eminently qualified for the careful, valuable study they have published.

GIDEON K. de FOREST

PRINCIPLES OF IMMUNOLOGY. By John E. Cushing and Dan H. Campbell. New York, McGraw-Hill Book Co., Inc., 1957. 344 pp. $6.50.

This work is a refreshing addition to the literature of immunology, one which recognizes for the first time the legitimate interests in immunological

principles of the student of general biology, who has been universally neglected in favor of the more specialized student of infectious diseases. It is designed primarily for the college senior or graduate student in biology; the references are intended to be illustrative of points requiring special emphasis rather than exhaustive in scope. The preface states: "Many controversial problems have been purposely omitted since the authors believe such discussions merely lead to confusion until the student has acquired a detailed knowledge of the entire field. Consequently the material presented here will be of little help to the advanced student or research worker in immunology except, perhaps at times, to offer a new perspective, at an elementary level, on some old problems."

Although repetition by restatement is a recognized pedagogic principle, the authors occasionally carry this to somewhat excessive lengths. Thus, the numerous serological reactions are each defined in a sentence or two in the opening pages, redefined, at paragraph length, in pages 29-40, and treated more definitively in chapters 13-15. The authors state that "very little of medical immunology is discussed," which would appear to be a modest understatement for the discussions included in chapters 10 and 15. Moreover, in a few sections, such as those on haptens, or the isolation of antibodies, the authors indulge mildly in the hobby-riding to which immunologists (present company included!) are so prone and which appears out of character with the announced purpose of the book. One wishes that the space gained from a judicious pruning of these sections on technical serology, to be found in any immunological text, could have been utilized for a presentation of the broader aspects of the host-parasite relationship so admirably developed in the writings of Theobald Smith or Sir Macfarlane Burnet, and which should be part of the education of all general biologists.

The excellence of the book lies, of course, in its positive aspects. The materials on the population genetics of antigens, serological taxonomy, antigenic individuality, fertilization, embryonic development of antigens, immunology in relation to differentiation, and comparative immunology, are highly in accord with its purpose and fully justify the price of admission ($6.50).

HENRY P. TREFFERS

THE CHEMISTRY AND BIOLOGY OF PURINES. G. E. W. Wolstenholme and C. M. O'Connor, Eds. Ciba Foundation Symposium. Boston, Little, Brown, and Co., 1957. xii, 327 pp. $9.00.

In view of the avalanche of papers dealing with the chemistry and biology of purines descending upon the many workers in this field the book under discussion is valuable indeed.

As in the previous volumes of the Ciba Foundation series, in which the proceedings of a Ciba Foundation sponsored international conference are presented in book form, a large number of subjects pertinent to the field is covered, each paper being followed by a frequently stimulating discussion and a brief bibliography.

The initial portion of the book is concerned with the organic and physical organic chemistry of purines. A partial list of contents includes topics as

diverse as the preparation and attempted preparation of some fluoropurines (A. Bendich *et al.*), new approaches to the synthesis of purines, purine-N-oxides, and pyrazolopyrazines (E. C. Taylor *et al.*), some new N-methylpurines (G. B. Elion), the structure of hydroxypurines (D. J. Brown, S. F. Mason), the π-electron properties of purine (S. F. Mason), the transformation of purines into pteridines (A. Albert), cyclonucleosides (D. M. Brown, A. Todd, and S. Varadarajan), and the stereochemistry of nucleoside synthesis (B. R. Baker).

The biological and biochemical part of this volume discusses among other things the pseudo vitamin B_{12} series (E. L. Smith, S. K. Kon), puromycin (B. L. Hutchings), 9-β-D-ribofuranosylpurine (G. B. Brown *et al.*), the role of one-carbon units in purine biosynthesis (G. R. Greenberg and L. Jaenicke), the *de novo* synthesis of inosinic acid (J. M. Buchanan *et al.*), the control of purine catabolism by xanthine oxidase (F. Bergel *et al.*), 8-azapurines (R. E. Matthews) and the effects of 6-mercaptopurine *in vitro* (G. B. Elion and G. H. Hitchings), and in human leukemia (D. A. G. Galton).

Certainly any reader of this book should be able to gain an excellent survey of some of the topics presently occupying the organic chemists, biochemists, and biologists working with this important group of compounds. This would be particularly true if the reader were familiar with the earlier Ciba Foundation volume *The Chemistry and Biology of Pteridines,* the chemistry and biochemistry of the purines and the pteridines being as closely related as they are.

HENRY G. MAUTNER

REGULATION AND MODE OF ACTION OF THYROID HORMONES. Ciba Foundation Colloquia on Endocrinology, Vol. X. G. E. W. Wolstenholme and Elaine C. P. Millar, Eds. Boston, Little, Brown and Company, 1957. 311 pp. $8.50.

At this colloquium on the regulation and mode of action of thyroid hormones, held 20-23 June 1956, some eighteen papers were presented concerning the present status of the thyroid hormone or hormones. There are three aspects to this problem of major interest: (i) what is the compound that acts in the body to produce the effects usually associated with the thyroid, if there is in reality a single substance, (ii) what factors regulate the output of this substance or its precursor from the thyroid, and (iii) what is the precise mechanism of action of the active compound or compounds at the cellular level? The papers presented consider these problems in some detail. It is quite clear that neither thyroxine nor triiodothyronine is the final active form of the hormone. The acetic acid derivatives of these, tetra and triiodothyroacetic acid (TETRAC and TRIAC) are more rapid in action and have been found in tissues, but there is not complete assurance that these are the final active forms of the hormones. With respect to the control of the liberation of thyroxine and triiodothyronine from the thyroid, it is clear that while the feed-back hypothesis involving the anterior pituitary and thyroid is attractive, it is not adequate to account for the complete regulation of thyroid activity. Finally, while there is unequivocal evidence for the action of thyroxine and related compounds influencing bio-

chemical processes at a cellular level, and in separated subcellular particles in the expected direction, the exact mechanisms involved are as yet unsolved. This complete and up-to-date source of information will be of great value to those interested in this field.

DESMOND D. BONNYCASTLE

ORTHOPEDIC SURGERY IN THE MEDITERRANEAN THEATER OF OPERATIONS. By Oscar P. Hampton, Jr., M.D., FACS, Colonel MC USAR. Washington, D. C., Department of the Army, 1957. 368 pp. illus.

This book gives a complete and comprehensive study of the combat-incurred injuries in the Mediterranean Theater (previously North African Theater) from November 8, 1942 to May 2, 1945. The studies which were carried out over this period produced changes in the concepts and methods of treatment of severe, compound, soft tissue and skeletal injuries. They are briefly as follows:

In the early period of the war the concept of treatment was plasma for shock, sulfanilimide drug for the prevention of infection, and the closed plaster (Trueta) management for fractures and soft tissue wounds. In the latter part of 1943 the management of these severe wounds was divided into four phases. The first phase being that of first aid which consisted of the arrest of hemorrhage, plasma, morphine, sterile dressings, and splinting of the part. The second phase consisted of the initial surgery at the forward hospital. It was here that resuscitation, whole blood transfusions, adequate initial debridement of the wounds, and dressing of the wounds with fine mesh gauze and splinting with plaster casts was instituted. The objectives at this stage were to save life, limbs, and to prevent infection. Fresh wounds were operated on as soon as possible and the patient transferred to a fixed hospital 24 to 48 hours later.

In phase three it was found that seven to ten days' post-wounding was the optimum time to carry out preparative surgery. This consisted of wound revision, of further debridement, closure of the wound, and fracture reduction by manipulation and plaster or skeletal traction immobilization. Internal fixation was employed only in special circumstances. It is interesting to note that the largest percentage of failures resulted from plating of tibial fractures. Dependent drainage with drains removed 3 to 10 days later was instituted in the majority of compounded wounds and fractures. It was here that dirty wounds were converted to clean fresh wounds by secondary excisional surgery and drainage of closed spaces.

Phase four consisted of the reconstruction procedures for the zone of interior hospitals.

Dr. Hampton has shown conclusively that the most important consideration of the management of compound fractures and wounds of joints in time of war was that of sound basic surgical principles.

A considerable period of time has elapsed since 1945 and our knowledge of antibiotic therapy, electrolyte and fluid balances, as well as new methods of internal fixation of fractures, has increased profoundly. Nevertheless, if and when we encounter mass casualties, the basic principles which were learned in this war should not be forgotten.

LELAND LUGAR

ADDITIONAL BOOKS RECEIVED

Doctor Courageous, the Story of Dr. Grantly Dick Read. By A. Noyes Thomas. New York, Harper & Bros., 1957. 218 pp. $3.75.

Meredith's Science of Health. By Warren H. Southworth and Arthur F. Davis. 3d ed. New York, McGraw-Hill Book Co., Inc., 1957. 481 pp. $5.00.

Nuclear Energy in the South. Redding S. Sugg, Jr., Ed. Baton Rouge, Louisiana State University Press, 1957. 151 pp. $3.50.

Citadel, Market and Altar; Emerging Society. Outline of Socionomy, the New Natural Science of Society. By Spencer Heath. Baltimore, The Science of Society Foundation, Inc., 1957. 259 pp. $6.00.

Fear: Contagion and Conquest. By James Clark Moloney. New York, Philosophical Library, 1957. 140 pp. $3.75.

Psychology, Evolution and Sex. By Cecil P. Martin. Springfield, Ill., Charles C Thomas, 1956. 166 pp. $4.75.

Manual of Nutrition. New York, Philosophical Library, 1957. 67 pp. $3.50.

Laboratory Manual of Microbiology. By H. Magdalene Steward. 2d ed. St. Louis, C. V. Mosby Co., 1957. 100 pp. $2.25.

Histology. By H. G. Q. Rowett. New York, Rinehart & Co., Inc., 1957. 47 pp. $.95.

Symposium on Biocolloids given at Research Conference for Biology and Medicine of the Atomic Energy Commission. Sponsored by the Biology Division Oak Ridge National Laboratory, Gatlinburg, Tennessee, April 12-14, 1956. Philadelphia, The Wistar Institute of Anatomy and Biology, 1957.

Current Contents of Pharmaceutical Publications. Philadelphia, Eugene Garfield Associates, 1957.

Translations of the Beltone Institute for Hearing Research. Charles Lightfoot, Ed. No. 7, *Our Determination of the Difference Limen for Intensity Modulation of Pure Tones, its Validity and its Topo-Diagnostic Efficiency, a translation of the German . . .* by E. Lüscher which appeared in *Acta Oto-Laryngologica* (Stockholm) vol. 45, pp. 402-415, 1955. Chicago, Ill., Beltone Institute for Hearing Research, 1957. 18 pp.

British Medical Bulletin, volume 13, number 3, September 1957. *Autonomic Nervous System.* London, The British Council, 1957. 230 pp. $3.25.

Heredo-Retinopathia Congenitalis. Monohybrida Recessiva Autosomalis. A Genetical-Statistical Study by Carl Henry Alström. Lund, 1957. 178 pp.

Viruses in Search of Disease. By Robert J. Heubner, W. W. Ackermann [and others]. Annals of the New York Academy of Sciences, vol. 67, art. 8, pp. 209-446. New York, 1957. $5.00.

Unstable Chemical Species: Free Radicals, Ions, and Excited Molecules. By Thomas Freund and Henry C. Thacher [and others]. Annals of the New York Academy of Sciences, vol. 67, art. 9, pp. 447-670. New York, 1957. $4.00.

Meprobamate and other Agents Used in Mental Disturbances. By James G. Miller and Frank M. Berger [and others]. Annals of the New York Academy of Sciences, vol. 67, art. 10, pp. 671-894. New York, 1957. $4.00.

The Effects of the Sulfonylureas and Related Compounds in Experimental and Clinical Diabetes. By Rachmiel Levine, I. P. Ackerman [and others]. Annals of the New York Academy of Sciences, vol. 71, art. 1, pp. 1-292. New York, 1957. $4.00.

CLINICAL experience in the treatment of respiratory tract infections with

SIGNEMYCIN* V

OLEANDOMYCIN TETRACYCLINE-PHOSPHATE BUFFERED

acute pharyngitis
pneumonia
pleurisy
otitis media
bronchitis
sinusitis
bronchiectasis
tonsillitis
influenza
bronchopneumonia
pansinusitis
laryngitis
tracheitis
ethmoiditis
streptococcal pharyngitis
nasopharyngitis
tracheobronchitis
bacterial pneumonia due to resistant pneumococci, staphylococci, or mixed flora
viral or nonspecific pneumonia not responsive to other therapy
lung abscess
follicular tonsillitis
pharyngitis caused by resistant staphylococci, Streptococcus viridans, or hemolytic Streptococcus
lobar pneumonia
viral URI

of **934** patients with respiratory infections treated with Signemycin†[1]

875 patients showed an excellent or good response

38 patients had fair response

21 patients had a poor response

and with outstanding safety and toleration **914** patients had no side effects

References: 1. Case reports in the Pfizer Medical Department Files from fifty-three clinicians, and the following published reports: Shubin, H.: Antibiotic Med. & Clin. Therapy 4:174 (March) 1957. Carter, C. H., and Maley, M. C.: Antibiotics Annual 1956-1957, New York, Medical Encyclopedia, Inc., 1957, p. 51. Winton, S. S., and Chesrow, E.: Ibid., p. 55. LaCaille, R. A., and Prigot, A.: Ibid., p. 19.

*Trademark
†Trademark, oleandomycin tetracycline

Increasing use of Signemycin V and other Signemycin formulations has confirmed the value of this agent in the armamentarium of the physician treating antibiotic-susceptible infections, particularly those seen at home or in office where susceptibility testing may not be practicable and where immediate institution of the most broadly effective therapy is necessary.

Pfizer

World leader in antibiotic development and production

PFIZER LABORATORIES, *Division, Chas. Pfizer & Co., Inc.*, Brooklyn 6, N. Y.

12,000,000 Orinase tablets stored at Upjohn's Kalamazoo plant.

12-million-tablet test

Before Upjohn released Orinase,* its new oral antidiabetic agent, in June of this year, the Company had conducted one of the most extensive clinical testing programs ever granted a new pharmaceutical product. More than 12,000,000 Orinase tablets were given to physicians for patient trials during 16 months prior to release date.

Orinase was used investigationally in more than 18,000 patients under the supervision of 1,200 physicians. Complete information was kept on 6,000 patients whose reactions were evaluated, with the assistance of automatic data processing equipment, on the basis of 29 criteria.

Upjohn left no stone unturned in its examination of this revolutionary new drug. Before release, Orinase had proved its usefulness in the conditions for which it is now being prescribed.

*TRADEMARK, REG. U. S. PAT. OFF. — TOLBUTAMIDE, UPJOHN

Upjohn

Medicine...
designed for health...
produced with care.